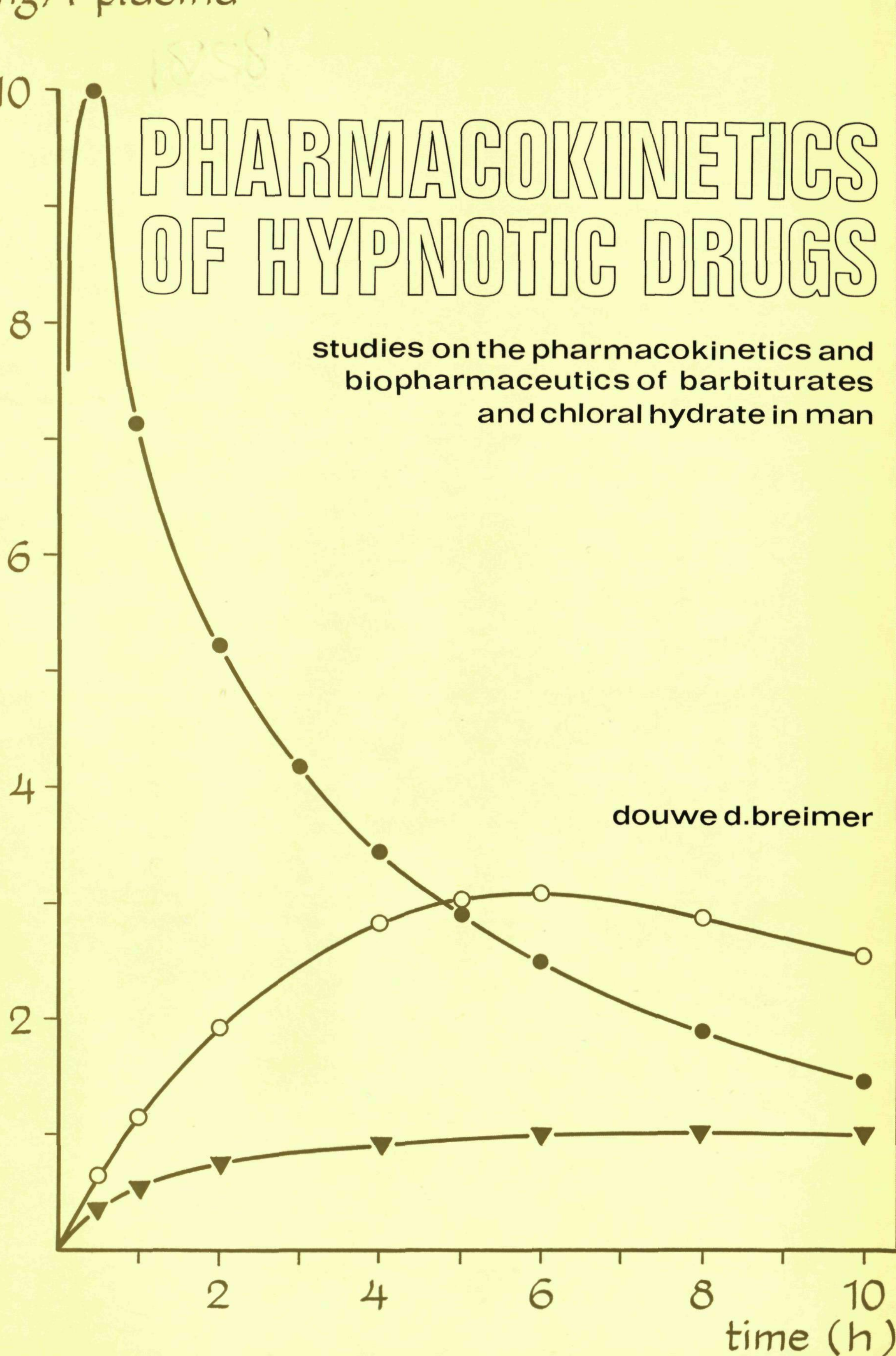
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PHARMACOKINETICS
OF HYPNOTIC DRUGS
studies on the pharmacokinetics and
biopharmaceutics of barbiturates
and chloral hydrate in man
douwe d.breimer

PHARMACOKINETICS OF HYPNOTIC DRUGS

STUDIES ON THE PHARMACOKINETICS AND BIOPHARMACEUTICS OF BARBITURATES AND CHLORAL HYDRATE IN MAN

PROMOTOR:

PROF. DR. J. M. VAN ROSSUM

PHARMACOKINETICS OF HYPNOTIC DRUGS

STUDIES ON THE PHARMACOKINETICS AND BIOPHARMACEUTICS OF BARBITURATES AND CHLORAL HYDRATE IN MAN

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voor joan, marieke, wietske en lianda

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CONTENTS

pag.

PHARMACOKINETIC SYMBOLS 12

PREFACE 13

SECTION I

SCOPE AND INTENT 15

CHAPTER 1 KINETICS OF DRUG DISTRIBUTION AND DRUG ELIMINATION 17

Introduction 17

Pharmacokinetic models 18

Plasma concentration during chronic medication 27

Capacity-limited elimination 28

The curve-fitting program "FARMFIT" 30

CHAPTER 2 BIOPHARMACEUTICS AND KINETICS OF DRUG ABSORPTION 33

Introduction 33

Kinetics of drug absorption 34

Bioavailability estimation 38

Pharmaceutical factors affecting bioavailability and absorption rate 40

Physiological factors affecting bioavailability and absorption rate 41

Effect of bioavailability and absorption rate on drug action 43

CHAPTER 3 HYPNOTIC DRUG THERAPY, PHARMACOKINETIC AND BIOPHARMACEUTICAL ASPECTS 49

Introduction 49

Pharmacokinetic aspects of hypnotic drug therapy 50

Biopharmaceutical aspects of hypnotic drug therapy 56

The investigations described in this thesis 57

pag.

SECTION II

ANALYTICAL PROCEDURE 61

CHAPTER 1 THE GAS CHROMATOGRAPHIC DETERMINATION OF BARBITURATES IN PLASMA 63

CHAPTER 2 HEXOBARBITAL IN PLASMA AND URINE 71

CHAPTER 3 3-KETO-HEXOBARBITAL IN URINE 79

CHAPTER 4 METHOHEXITAL IN PLASMA 86

CHAPTER 5 VINYLBITAL IN PLASMA AND URINE 90

CHAPTER 6 HEPTABARBITAL IN PLASMA AND URINE 95

CHAPTER 7 BUTOBARBITAL IN PLASMA 100

CHAPTER 8 CYCLOBARBITAL IN PLASMA 104

CHAPTER 9 CHLORAL HYDRATE, TRICHLOROETHANOL, TRICHLOROETHANOL-GLUCURONIDE AND TRICHLOROACETIC ACID IN BLOOD AND URINE, EMPLOYING GAS CHROMATOGRAPHY WITH HEAD-SPACE ANALYSIS 109

SECTION III

PHARMACOKINETICS AND BIOPHARMACEUTICS OF BARBITURATES AND CHLORAL HYDRATE 119

CHAPTER 1 HEXOBARBITAL AND HEXOBARBITAL SODIUM 121

A. General introduction 121

B. Intravenous administration 123

C. Oral and rectal administration 134

CHAPTER 2 (+)- AND (—)-HEXOBARBITAL 156

A. Pharmacokinetics in man 156

B. Pharmacokinetics in rats and in the isolated perfused rat liver 166

CHAPTER 3 METHOHEXITAL, INTRAVENOUS AND ORAL ADMINISTRATION 185

pag.

CHAPTER 4 VINYLBITAL, ORAL AND RECTAL ADMINISTRATION 196

CHAPTER 5 HEPTABARBITAL AND HEPTABARBITAL SODIUM, ORAL ADMINISTRATION 213

CHAPTER 6 BUTOBARBITAL, ORAL ADMINISTRATION 230

CHAPTER 7 CYCLOBARBITAL CALCIUM, ORAL ADMINISTRATION 240

CHAPTER 8 CHLORAL HYDRATE 250

A. General introduction 250

B. Pharmacokinetics and metabolism 252

C. Biopharmaceutics 268

1. Rectal administration 268

2. Oral administration 277

SECTION IV

PHARMACOKINETICS AND LIVER DISEASE 289

CHAPTER 1 PHARMACOKINETICS OF HEXOBARBITAL IN PATIENTS WITH LIVER DISEASE 291

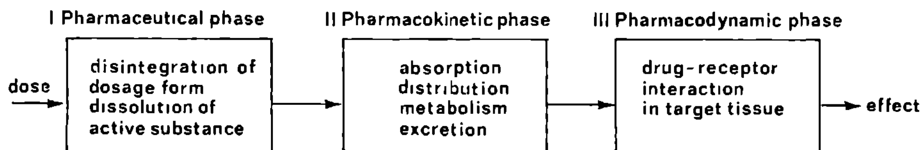
CURRICULUM VITAE 311

PHARMACOKINETIC SYMBOLS

t	time (min or h)
τ	time constant (min or h)
T	zero-order infusion time (min or h)
r	rate constant (1/min or 1/h)
C	concentration (mg/l or μg/ml)
Q	amount (mg)
A	coefficient (mg/l or μg/ml)
k	clearance constant (ml/min or l/h)
V	volume (ml or l)
F	bioavailability ($0 \leqslant F \leqslant 1$)

In most industrialized countries hypnotic drugs are very widely prescribed for the treatment of insomnia. Several years ago it was estimated that in the United Kingdom alone 22 million prescriptions for hypnotic drugs were prescribed per annum. A rough estimate indicates that about one night's sleep in every ten was hypnotically induced in this country. There is no reason to believe that these figures are very different for those of other western countries or that these figures have decreased subsequently. Although insomnia is usually not regarded as an illness in itself, but as a symptom of an underlying physical or emotional disorder, the relief of this symptom may require adequate hypnotic drug treatment.

After administration of a drug product to man, three main phases can be distinguished, as depicted in the following scheme:



During the pharmaceutical phase the disintegration of the dosage form and the dissolution of the active ingredient in the surrounding fluids are the pertinent factors. Biopharmaceutics is the study of the influence of formulation factors on the therapeutic activity of a drug product. This is generally assessed by measuring the extent (bioavailability) and the rate (absorption rate) at which the dosage form delivers the active substance to the general circulation. Also, physiological factors such as the metabolism of a drug during first passage through the liver are important in this respect. The pharmacokinetic phase comprises the kinetics of absorption, distribution, metabolism and excretion of a drug. The time-course of drug concentrations in plasma and other body-fluids is studied, so that certain important parameters can be calculated. In the pharmacodynamic phase the actual interaction between the drug in the target tissue and the receptor occurs, which results in the pharmacological effect. The various processes which take place during the pharmaceutical and pharmacokinetic

phase mainly determine the onset and duration of drug action. Only for a few hypnotic drugs have the pharmacokinetics been well defined, whereas data for the influence of formulation factors on bioavailability and absorption rate (biopharmaceutics) are almost completely lacking.

The investigations described in this thesis have been initiated since information concerning these aspects is pertinent to rational hypnotic drug therapy. Several barbiturates and chloral hydrate, incorporated in different pharmaceutical formulations and administered by various routes, have been investigated. The results have been discussed in relation to the utility of the various compounds and dosage forms for their use in general practice. In addition, the pharmacokinetics of hexobarbital, in patients with liver disease, were studied in order to obtain information concerning possible changes in the kinetic behaviour of a drug under pathological conditions.

SECTION I

SCOPE AND INTENT

CHAPTER 1

KINETICS OF DRUG DISTRIBUTION AND DRUG ELIMINATION

INTRODUCTION

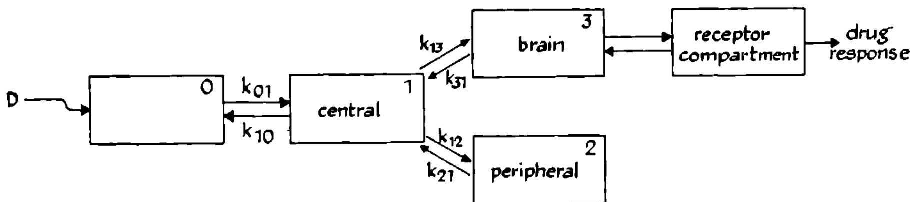
It is a common belief that drugs produce their pharmacological effect in biological systems by reacting with receptor sites, which are located in the target tissues. The intensity of effect of reversibly acting drugs depends on the degree of receptor occupation, which, in turn, is determined by the concentration of the drug in the direct environment of the receptors (biophase) and the affinity of the drug for the receptors (Ariëns, 1964). Usually, it is not possible to determine drug concentrations at the receptor sites in man, since these are not accessible for sampling. However, all tissues are supplied with plasma and it is obvious that a certain relationship exists between drug concentration in plasma and the concentration in the biophase, although such a relationship may be complex. The plasma is easily accessible for sampling. Following the administration of a drug to man or animal, several processes take place: 1. *absorption* from the site of application to the plasma; 2. *distribution* from the plasma into organs and tissues, and 3. *elimination* by biotransformation (e.g. in the liver) or by excretion (e.g. through the kidney). As a consequence of these events, which partly occur simultaneously, the drug concentration in plasma changes with time. Likewise, the concentration in the biophase also changes and so does the pharmacological effect. In other words, changes in the time course of drug concentrations in plasma will affect the time course of drug action. It is for this reason that information on the kinetics of a drug in the body is of great interest in pharmacology. Pharmacokinetic data will help to optimize drug therapy, with respect to the choice of the proper drug and drug preparation, as well as with respect to a proper dosage regimen (van Rossum, 1971).

Pharmacokinetics deals with the kinetics of absorption, distribution, metabolism and excretion of drugs and other substances in man or animals. Its purpose is to study the time course of drug concentrations in plasma and other fluids, tissues and excreta and to construct models suitable to interpret such

data (Wagner, 1971). The relationship between pharmacological response and concentrations of drugs or their metabolites in body fluids is also pertinent to pharmacokinetics (Levy and Gibaldi, 1972; Levy, 1973). Several reviews on this topic have appeared in the literature (Dost, 1968; Gibaldi, 1968; Wagner, 1968; Portmann, 1970; van Rossum, 1971; Wagner, 1971; Notari, 1971).

PHARMACOKINETIC MODELS

One of the basic tools of science is the use of models to simulate and simplify real systems. In physiology and pharmacokinetics compartment models are most often used to describe the behaviour of endogenous substances or exogenous substances, including drugs (Riggs, 1963; Rescigno and Segre, 1966). These models assume that the biological system can be described as one or more connected pools, in which an amount of drug may be homogeneously distributed throughout an apparent volume of distribution. The transfer of drug between compartments is usually assumed to proceed by an apparent first-order process. In Scheme 1 a representation is given of the processes taking place after administration of a drug to the body. These involve consecutive and simul-



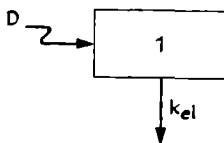
SCHEME 1

taneous competing rate processes or clearance processes, where clearance may be thought of as that volume of the total volume of a certain compartment which is totally cleared of drug per unit of time. The clearance constants (symbol k), which have the dimensions of flow constants (ml/min), govern the drug transport between the compartments and the environment. The rate constants (symbol r), or time constants (symbol τ), which are a function of the clearance constants and the volumes of the compartments, determine how long and in what concentration the drug remains in the various compartments, including the receptor compartment. It is evident that the model as depicted in

Scheme 1, is a rather complicated one, although it may approximate a real situation. In practice, sampling will generally be restricted to the central or plasma compartment, thus it may be impossible to obtain sufficient data for such a complex model to have any utility. For this reason, the simplest model by which the experimental data can be explained is the one of choice. Such a model is, of necessity, based upon specific assumptions, which must be recognized and tested, whenever possible, by experimental data (Mayersohn and Gibaldi, 1971; Nooney, 1966; Westlake, 1971). The relatively simple models which are commonly used in pharmacokinetics and which are relevant to the investigations described in this thesis, will be discussed briefly. If necessary, further extensions will be made in this or the following Sections. For instance, the kinetics of drug absorption will be discussed separately in Chapter 2, Section I.

1. The single-compartment open model

When the rate of drug transfer over the various fluids and tissues of the body greatly exceeds the rate of elimination, the body may be considered as a single open compartment (Scheme 2). Generally the elimination of drugs from the



SCHEME 2

body occurs according to a first-order process and the elimination rate can be described by the following equation:

$$\frac{dQ}{dt} = -k_{el}\,C_p \quad \text{or} \quad \frac{dC}{dt} = -\frac{k_{el}}{V_f}\,C_p \qquad (1)$$

where dQ/dt is the elimination rate (mg/min), k_{el} the elimination clearance constant (ml/min) and C_p the plasma concentration (mg/l). Elimination is considered as a clearance from a single compartment with a volume V_f (l). The concentration of the drug in the various parts of the body may be quite different, so that the volume of the compartment does not necessarily reflect a physical reality. It is called therefore an apparent volume of distribution, which is derived from the concentration of drug in plasma. Usually the total plasma

concentration is determined, including the protein bound fraction; association and dissociation of the drug-protein complex are assumed to occur very rapidly as compared to distribution and elimination and are also assumed to be describable by linear differential equations (Schoenemann et al., 1973). Integration of eq. (1) leads to the following equation for the plasma concentration:

$$\ln C_p = \ln A - t/\tau_{el} \quad \text{or} \quad C_p = A\, e^{-t/\tau_{el}} = A\, 2^{-t/t_{1/2}} \tag{2}$$

where A is the plasma concentration at $t = 0$, τ_{el} the elimination time constant and $t_{1/2}$ the biological half-life. Furthermore it can be derived that:

$$\tau_{el} = V_f/k_{el} \quad \text{and} \quad t_{1/2} = \ln 2 \,.\, \tau_{el} = 0.69\, \tau_{el} \tag{3}$$

From eq. (2) it follows that the logarithm of the plasma concentration decreases linearly with time. Theoretical examples are shown in Fig. 1 and it is evident that semi-logarithmic plots give straight lines for single-compartment first-order kinetics. The biological half-life can easily be deduced from this type of curves, since it is the time required to reduce any given plasma concentration to 50%.

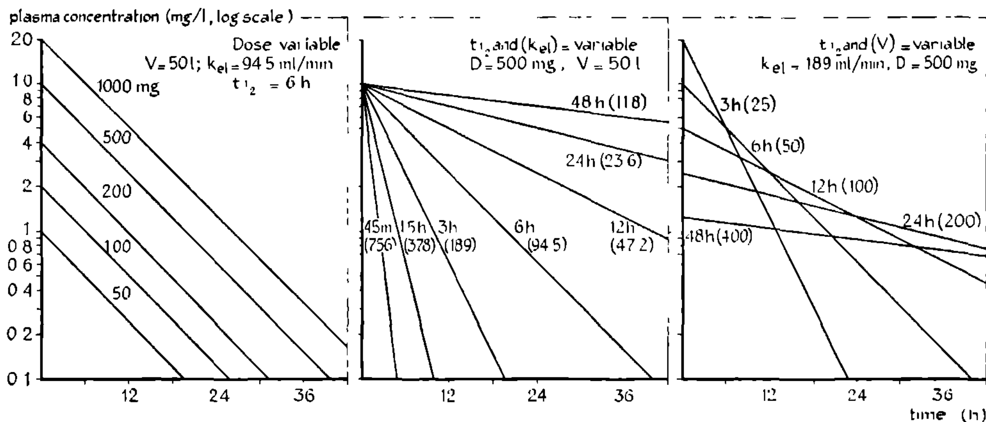


FIG. 1. Theoretical plasma concentration curves based upon single-compartment kinetics after rapid i.v. injection. Straight lines are obtained on semi-logarithmic scale. *Left:* A variation in the administered dose leads to a proportional change in the zero-time concentration and a parallel shift of the curves. *Middle:* From the slope of the curves the biological half-life can be determined. A decrease of the elimination clearance (in parentheses) causes a proportional increase in the half-life. *Right:* A decrease in the volume of distribution (in parentheses) leads to a proportional decrease of the zero-time concentration and to a proportional increase of the half-life.

This parameter is generally used in practice to characterize the rate of elimination of a certain drug from the body. Many of these have been summarized recently by Ritschel (1972). A long half-life does not necessarily imply a low clearance. A drug with a high clearance value may still exhibit a large half-life when the volume of distribution is large, e.g. amphetamines (Rowland, 1969; Vree, 1973). V_f can be obtained from the intercept of a semi-logarithmic plasma concentration curve at zero-time (V_f = A/Dose). It should be emphasized that it is not the biological half-life that characterizes the actual elimination process of a drug from the body, but the clearance constant (van Rossum, 1971). The total body clearance (k_{el}) is the sum of the clearance constants representing the various metabolic pathways, renal excretion and possibly other elimination mechanisms (saliva, sweat, bile, lungs). In formula:

$$k_{el} = k_m + k_r + \ldots\ldots \quad (4)$$

where k_m is the metabolic and k_r is the renal clearance constant. Since elimination of most hypnotic drugs occurs by biodegradation, the total body clearance of the compounds used in the present investigations generally equals the metabolic clearance.

2. *The two-compartment open model*

Distribution of a drug throughout the various tissues in the body is, in many instances, not extremely rapid compared to the elimination process. In the two-compartment open model it is assumed that there is a central compartment, consisting of plasma and other fluids and tissues, which is instantaneously accessible to the drug. The second or peripheral compartment consists of fluids and tissues which are more slowly accessible to the drug. On the basis of similarities in blood flow and tissue-plasma partition coefficients for a certain drug, various tissues may be grouped together, such that the two compartments may have some physiological meaning (Riegelman et al., 1968). This may vary substantially from drug to drug, since the physico-chemical properties of a drug mainly govern the extent and the rate of distribution in the body. For instance in the case of the anaesthetic barbiturates (thiopental, hexobarbital, methohexital) the brain is likely to be classified with the central compartment, whereas for less lipophilic barbiturates the brain may belong to the second compartment. The two-compartment model is depicted in Fig. 2 and it is assumed that elimination takes place from the central compartment. The different rate processes can be described by the following differential equations:

$$\frac{dQ_1}{dt} = -k_{el} C_1 - k_{12} C_1 + k_{21} C_2 \tag{5}$$

$$\frac{dQ_2}{dt} = -k_{21} C_2 + k_{12} C_1 \tag{6}$$

$$\frac{dQ_{el}}{dt} = k_{el} C_1 \tag{7}$$

where Q_1 and Q_2 is the amount (mg) and C_1 and C_2 is the concentration (mg/l) in compartment 1 and 2 respectively, Q_{el} is the amount eliminated (mg), k_{12} and k_{21} are the clearance constants (ml/min) for distribution and k_{el} is the elimination clearance constant (ml/min). Usually sampling can only be performed in the central compartment, to which the plasma belongs. Integration of eq. (5) gives the concentration in this compartment (plasma concentration C_p):

$$C_p = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} \tag{8}$$

where A_1 and A_2 are the coefficients (mg/l) and τ_1 and τ_2 are the time constants (min), which now depend on the volume of the first (V_1) and the second (V_2) compartment (l), as well as on all clearance constants (Riegelman et al., 1968; Gibaldi, 1969). In general the biological half-life is calculated from the largest time constant ($t_{1/2} = 0.69\ \tau_2$). The plasma concentration - time curve, which can be described mathematically by eq. (8), is characterized by a biphasic semi-logarithmic plot. Such a time course is found frequently after i.v. administration of a drug, which indicates that two-compartment kinetics may be valid. In Fig. 2 a typical example is given for hexobarbital after i.v. infusion to a healthy volunteer. From the linear part of the post-infusion curve τ_2 and A_2 can be deduced, whereas τ_1 and A_1 can be estimated by the feathering or subtraction method. In this particular instance the experimental values for A_1 and A_2 are not the true coefficients for i.v. bolus injection and have to be corrected for the duration of infusion (Chapter 1, Section III). Once the four experimental parameters have been determined, the important pharmacokinetic parameters for a drug in the body may be calculated from the following equations (van Rossum, 1971):

$$V_1 = D/(A_1 + A_2) \tag{9}$$

$$k_{el} = D/(A_1\tau_1 + A_2\tau_2) \tag{10}$$

$$r_{12} = \frac{k_{12}}{V_1} = \frac{A_1A_2(\tau_2 - \tau_1)^2}{(A_1 + A_2)(A_1\tau_1 + A_2\tau_2)\tau_1\tau_2} \tag{11}$$

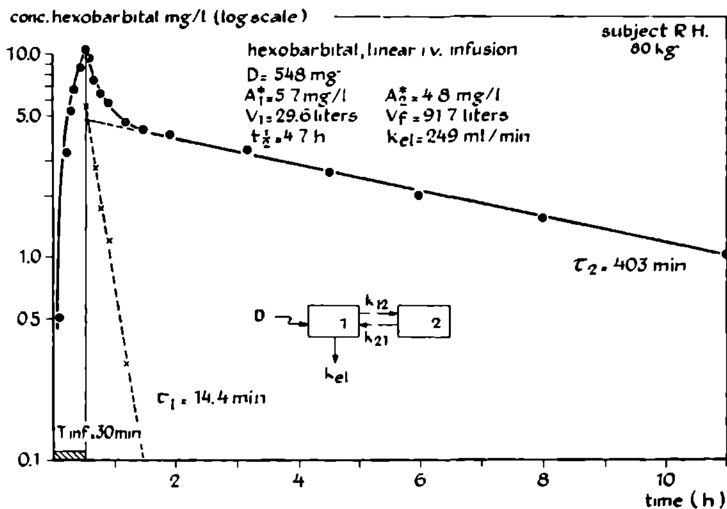


FIG. 2. Plasma concentration curve on semi-logarithmic scale following a zero-order infusion of 600 mg hexobarbital sodium (equivalent to 548 mg of the free acid) to a human volunteer. After termination of the infusion the shape of the curve is typical for the two-compartment open model (see scheme). The dotted line, obtained by the subtraction method, represents the first term of the two-term exponential equation. This rapid decline is mainly due to the rapid distribution of the drug over the various tissues. The terminal straight line represents the second term, which is due to the elimination of the drug. The biological half-life may be deduced from the slope of this line. Furthermore, the apparent volume of distribution (V_f) and the total body elimination clearance constant (k_{el}) can be calculated.

$$r_{21} = \frac{k_{21}}{V_2} = \frac{A_1\tau_1 + A_2\tau_2}{(A_1 + A_2)\,\tau_1\tau_2} \tag{12}$$

$$V_f = V_1\left(1 + \frac{r_{12}}{r_{21}}\right) = \frac{D\,(\tau_1^2 A_1 + \tau_2^2 A_2)}{(\tau_1 A_1 + \tau_2 A_2)^2} \tag{13}$$

where r_{12} and r_{21} are the distribution rate constants (1/min). Also, k_{12} may be calculated, although this is not possible for k_{21} since the volume of the second compartment cannot be estimated.

3. The three-compartment open model

For a drug which acts on the central nervous system, the body may be considered as a three-compartment open system. In Fig. 3A the model is depicted,

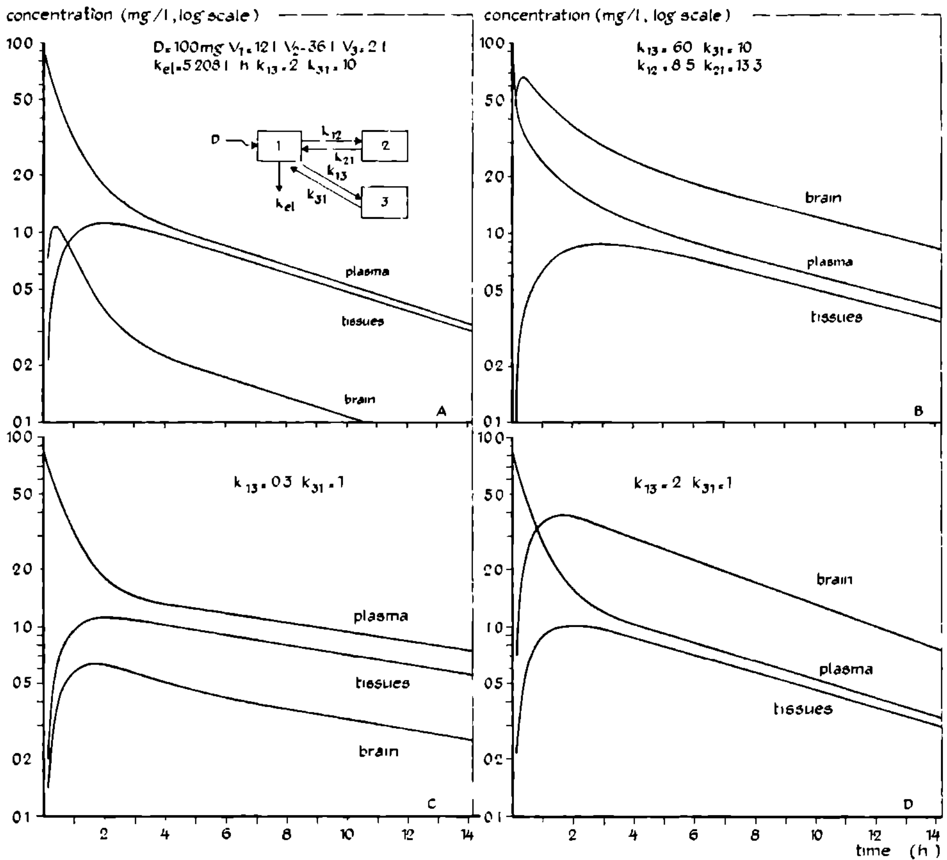


FIG. 3. Example of a three-compartment open model (see scheme in *A*), with theoretical curves representing drug concentration in plasma (compartment 1), in peripheral tissues (compartment 2) and in the brain (compartment 3). The pharmacokinetic parameters underlying these curves are given in *B*. The clearance constants governing drug entry into the brain and from the brain (k_{13} and k_{31}) are varied and the influence on the brain concentration and on the concentrations in the other two compartments is shown. It may be observed that the brain concentration may vary considerably despite comparable plasma concentrations.

where a central compartment (plasma) is equilibrating with the brain compartment and with a peripheral tissue compartment. In Fig. 3 a set of theoretical concentration curves are given in order to show the influence of the clearance constants governing the entry of drug into the brain and from the brain upon the brain concentration time-course. It is evident that the brain concentration may differ considerably, despite a constant elimination clearance. The rela-

tionship between plasma concentration and concentration in the brain may therefore be very complicated, although an unambiguous relationship exists. If a drug, however, is slowly eliminated compared to the distribution process, a steady state condition will result after some time and the brain concentration parallels the plasma concentration (Fig. 3).

For a three-compartment model the different rate processes can be described by the following differential equations:

$$\frac{dQ_1}{dt} = -(k_{el} + k_{12} + k_{13})\, C_1 + k_{21}C_2 + k_{31}C_3 \quad (14)$$

$$\frac{dQ_2}{dt} = k_{12}C_1 - k_{21}C_2 \quad (15)$$

$$\frac{dQ_3}{dt} = k_{13}C_1 - k_{31}C_3 \quad (16)$$

$$\frac{dQ_{el}}{dt} = k_{el}C_1 \quad (17)$$

where Q_3 is the amount (mg) and C_3 is the concentration (mg/l) in the third compartment, Q_{el} is the amount eliminated (mg) and k_{13} and k_{31} are the distribution clearance constants (ml/min) between the central and the third compartment. For the concentration in the central compartment (plasma concentration) the following equation is obtained after integration:

$$C_p = A_1\, e^{-t/\tau_1} + A_2\, e^{-t/\tau_2} + A_3\, e^{-t/\tau_3} \quad (18)$$

where τ_1, τ_2 and τ_3 are the time constants (min) and A_1, A_2 and A_3 are the coefficients (mg/l). These parameters may be deduced from the experimental curve and then the elimination clearance constant and the apparent volume of distribution can be calculated:

$$k_{el} = \frac{D}{A_1\tau_1 + A_2\tau_2 + A_3\tau_3} \quad (19)$$

$$V_f = \frac{D\,(A_1\tau_1^2 + A_2\tau_2^2 + A_3\tau_3^2)}{(A_1\tau_1 + A_2\tau_2 + A_3\tau_3)^2} \quad (20)$$

Obviously, in practice it is only necessary to consider the body as a three-compartment open system, when the experimental data require such an as-

sumption, e.g. when the plasma concentration - time curve exhibits three distinct phases after i.v. injection. It is often very difficult to distinguish between a two- and a three-compartment model (refer to methohexital, Chapter 3, Section III). Even in the theoretical plasma concentration curves of Fig. 3, which are based upon the three-compartment model, the three phases are difficult to unravel. The model may be valid, for instance, in the case of thiopental kinetics, where the equilibrium between plasma concentration and adipose tissue is reached very slowly (Price et al., 1960; Bischoff and Dedrick, 1968). Many experimental data are required to permit a reliable fit to a three-exponential equation. Details of the mathematics underlying the three-compartment model have been given by Skinner et al. (1959).

4. *Multi-compartment kinetics*

As indicated earlier, the body should in principle be regarded as a multi-compartment system, although in many cases it is not possible to distinguish between the separate compartments. This is mostly due to the limitations of the experimental procedure, which is especially true for human investigations. Nevertheless it is of interest to give a general equation for the plasma concentration, which is valid if all the processes involved are first-order, including absorption, distribution and elimination:

$$C_p = \sum_{i=1}^{n} A_i \, e^{-t/\tau_i} \tag{21}$$

where n is the number of compartments involved, A_i the *i*th coefficient (mg/l) and τ_i the *i*th time constant (min). In the case of oral or rectal administration n is the sum of compartments including the gastro-intestinal tract. The calculation of the pharmacokinetic parameters may be generalized:

$$V_1 = D \Big/ \sum_{i=1}^{n} A_i \tag{22}$$

$$k_{el} = D \Big/ \sum_{i=1}^{n} A_i \tau_i \tag{23}$$

$$V_f = D \sum_{i=1}^{n} A_i \tau_i^2 \Big/ \Big(\sum_{i=1}^{n} A_i \tau_i \Big)^2 \tag{24}$$

If elimination occurs from the central compartment by a concentration independent clearance process, then the following is also generally valid (Dost, 1968):

$$\frac{dQ_{el}}{dt} = k_{el} C_p = k_{el} \sum_{i=1}^{n} A_i e^{-t/\tau_i} \qquad (25)$$

where Q_{el} is the amount eliminated (mg). Obviously, the amount of drug eliminated after sufficiently long periods of time ($t \gg \tau_i$'s) equals the amount that was introduced into the body. Therefore integration of eq. (25) gives:

$$Q_{el} = k_{el} \int_0^{\infty} C_p dt = k_{el} . AUC \qquad (26)$$

where AUC is the area under the plasma concentration curve from $t = 0$ to $t = \infty$ (mg min/l). Eq. (26) is important in bioavailability studies, since often the area under the curve can be determined, whereas the exact time constants and coefficients cannot If a drug is not given by i v injection, but orally or rectally, it is possible that only a fraction F of the administered dose reaches the general circulation. This fraction (bioavailability) can be estimated from the area under the plasma concentration curve after oral or rectal administration, provided that the total body clearance has been determined via other independent measurements (i.v. injection). In formula·

$$F . D = k_{el} \ AUC \quad \text{or} \quad F = k_{el} . AUC/D \qquad (27)$$

The bioavailability of a drug depends to a large extent on the pharmaceutical formulation in which it is incorporated. This aspect, together with the kinetics of drug absorption, will be discussed in detail in Chapter 2, Section I.

PLASMA CONCENTRATION DURING CHRONIC MEDICATION

Often, drugs may be prescribed for longer periods of time, e g. antiepileptic agents and antibiotics are given on a multiple dosage regimen in order to maintain the plasma concentration above a minimal effective concentration. Hypnotic drugs are frequently taken each night, but in this case the plasma concentration should be above the effective concentration only during the night. Obviously,

certain limitations on the kinetics of a drug are exacted in order to be suitable in hypnotic therapy, as will be outlined in Chapter 3, Section I.

If a drug is administered repetitively with constant dosage intervals (Δt), then the plasma concentration at any time after the commencement of the therapy may be described, irrespective of the complexity of the kinetic models involved, (provided linear differential equations are valid) by the following equation (van Rossum and Tomey, 1970):

$$C_p = \sum_{i=1}^{n} A_i \frac{1 - e^{-j\Delta t/\tau_i}}{1 - e^{-\Delta t/\tau_i}} \cdot e^{-t/\tau_i} \qquad (28)$$

where n is the number of compartments involved, A_i is th *i*th coefficient, τ_i the *i*th time constant and *j* denotes the *j*th dose that has been administered, while t is the time after administration of the *j*th dose. In the case of single-compartment kinetics after oral administration, eq. (28) is reduced to the following (van Rossum, 1968; van Rossum and Tomey, 1968):

$$C_p = A \left[\frac{1 - e^{-j\Delta t/\tau_{el}}}{1 - e^{-\Delta t/\tau_{el}}} \cdot e^{-t/\tau_{el}} - \frac{1 - e^{-j\Delta t/\tau_a}}{1 - e^{-\Delta t/\tau_a}} \cdot e^{-t/\tau_a} \right] \qquad (29)$$

where A is the extrapolated zero-time concentration (mg/l) after the first oral dose, τ_{el} is the elimination time constant and τ_a is the absorption time constant. Once A, τ_{el} and τ_a have been determined for a certain drug, usually after a single oral dose, the theoretical plasma profile during repetitive medication can be calculated. This was accomplished for vinylbital (Chapter 4, Section III, Fig. 7) and butobarbital (Chapter 6, Section III, Fig. 5). For most drugs absorption occurs more rapidly than elimination and if τ_{el} is greater than Δt, substantial accumulation may occur until a certain plateau concentration is reached (see butobarbital). If τ_{el} is smaller than Δt, no real accumulation will become apparent and the plasma concentration fluctuates considerably during chronic medication. This was shown for instance in the case of heptabarbital (Chapter 5, Section III, Fig. 6) with a half-life of about 7 h and a dosage interval of 24 h. It is evident that the accumulation of a hypnotic drug in the body is undesirable.

CAPACITY-LIMITED ELIMINATION

Generally all processes in pharmacokinetics are assumed to be first-order, so that the rate of elimination of a drug may be directly proportional to the

drug concentration in the plasma. The differential equations describing drug transfer are linear under such conditions. The proportionality factor for the elimination process is the clearance constant (k_{el}), which is the parameter that characterizes the elimination of a drug from the body. Many drugs are eliminated in the liver by metabolism where certain enzymes are involved. Understandably, the rate of metabolism may be limited by the capacity of these enzymes. If these are saturated, then elimination becomes a zero-order process as has been shown for ethanol (Lundquist and Wolthers, 1958) and for part of salicylic acid metabolism (Levy et al., 1972). If biotransformation proceeds via a single pathway, then the rate of elimination may be described by use of a Michaelis-Menten equation (Levy et al., 1972; Wagner, 1973; van Ginneken et al., 1974):

$$\frac{dQ}{dt} = -\frac{\dot{Q}_m}{(K_m + C)} \cdot C \tag{30}$$

where $\dot{Q}_m$ is the metabolic capacity of the enzymes (mg/h) and is related to V_{max} when only one enzyme is involved. K_m is the apparent Michaelis-Menten constant (mg/l). It is assumed that the substrate concentration (C) in the clearance tissue is in equilibrium with the plasma concentration. If C equals the plasma concentration (C_p), it follows from eq. (30) and eq. (1) that:

$$k_{el} = \frac{\dot{Q}_m}{K_m + C_p} \tag{31}$$

For low plasma concentrations ($C_p \ll K_m$) the metabolic clearance is a simple function of the enzymatic constants which govern biotransformation:

$$k_{el} = \dot{Q}_m/K_m \tag{32}$$

Since the plasma concentration is directly related to the administered dose, increasing doses will ultimately lead to a concentration dependent metabolic clearance. If $C_p \gg K_m$ it will be clear that dQ/dt equals $\dot{Q}_m$, which implies that elimination proceeds as a zero-order process. In practice a capacity-limited elimination may often occur simultaneous with a non-saturated pathway (e.g. excretion into the urine). The kinetics are more complicated in such a case, which has recently been discussed by van Ginneken et al. (1974). In pathological conditions, e.g. liver disease (Section IV), the capacity of the liver enzymes may be far lower than for normal circumstances. In such a case k_{el} will decrease and elimination will be retarded. Also, if K_m decreases saturation

will occur at lower plasma concentrations and capacity-limited elimination may be encountered already at lower plasma levels.

THE CURVE-FITTING PROGRAM "FARMFIT"

The pharmacokinetic parameters according to the various models, may be fitted to the experimental plasma concentrations. For this purpose use is made of the non-linear least squares curve fitting program FARMFIT, which was developed by Drs. G. Fast of the Computer Centre, University of Nijmegen. The program finds a least squares fit to a set of concentrations $C(t_i)$ at times t_i to a model function $F(t)$, i.e. the program finds such values for the parameters that the sum of squares:

$$K = \sum_{(i)} \{C(t_i) - F(t_i)\}^2 \tag{33}$$

is minimized, assuming the same accuracy for the concentration data $C(t_i)$. If the concentration data $C(t_i)$ have different (absolute) errors of measurement e_i, then weights g_i inversely proportional to e_i are assigned to the data and the sum of squares:

$$K = \sum_{(i)} g_i^2 \{C(t_i) - F(t_i)\}^2 \tag{33a}$$

is minimized. It is supposed that 1. no systematic errors exist in the measurements, 2. the model function fits.

Because the calculation of the least squares fit is straightforward for linear parameters, the function F is split up:

$$F(t;A,\tau) = f_0(t;\tau) + \sum_{(j)} A_j f_j(t;\tau) \tag{34}$$

where A is the vector of the unknown linear parameters A_j with $j = 1,2,\ldots$ and τ is the vector of the unknown non-linear parameters τ_k with $k = 1,2,\ldots$ The function f_0 may be identically zero. The separate f-functions are generally not dependent on all non-linear parameters τ_k. If F only depends on linear parameters A_j, then it can be written as:

$$F(t;A) = f_0(t) + \sum_{(j)} A_j f_j(t) \tag{35}$$

where each f_j is independent of A_j. Then the least squares estimates of the A_j are the solutions of the linear system of equations:

$$\frac{\delta K}{\delta A_j} = 0 \quad \text{(normal equations)}, \tag{36}$$

where K is the sum of squares (33) or (33a) after substitution of (35).

If F is also dependent on non-linear parameters, a modification of the iterative method of Gauss-Newton is applied. Assuming that a set of parameters τ° is not far removed from the real solution τ, so that the difference $\Delta = \tau - \tau^\circ$ is small, then $F(t;A,\tau)$ can be approximated by a truncated Taylor series:

$$F(t;A,\tau) = F(t;A,\tau^\circ) + \sum_{(k)} \frac{\delta F(t;A,\tau^\circ)}{\delta \tau_k} \Delta_k \tag{37}$$

After substituting into (33) or (33a) the sum of squares K can be minimized with respect to Δ_k in the same way as with respect to A_j if F(t) has the form (35) because of the linearity in Δ.

For given data points with given weights and for given τ the linear parameters A_j can be solved directly via the normal equations $\delta K/\delta A_j = 0$, where K is the sum of squares (33) or (33a) after substitution of (34). So these solutions for A can be considered as functions $A(\tau)$ of τ. This is done by the program FARMFIT for the A_j in (37). Hence for the partial differentiation the function F is only considered as a function with non-linear parameters. The derivatives $\delta F/\delta \tau_k$ are calculated numerically. After determining the improved values $\tau_j^\circ + \Delta_j$ for the non-linear parameters the linear parameters are adapted to these improved values via the normal equations.

After a maximum number of repetitions of this process or after an increase of K, use is made of an interpolation method. If τ^1 and τ^2 are the last values obtained for the τ-vector of non-linear parameters, then the line connecting the points τ^1 and τ^2 in the τ-space is considered. The interpolation process searches for the optimum τ on this line, where the linear parameters A_j are always adapted via the normal equations. After such an iteration - interpolation cycle the resulting τ_j-values are compared with the τ_j-values from the previous cycle. If the variations of the τ_j-values are sufficiently small the program either finishes or continues with a larger accuracy.

The program also calculates estimates for the asymptotical covariance matrix and standard errors of the parameters A_j and τ_k. It may be expected that these estimates are incorrect if few concentration data are available and large errors of measurement are involved.

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CHAPTER 2

BIOPHARMACEUTICS AND KINETICS OF DRUG ABSORPTION

INTRODUCTION

Drugs are generally administered as drug products, which are referred to as dosage forms or drug delivery systems. During the last decade it has become apparent that drug products which contain the same amount of active drug, but which have been prepared in different ways, or contain different excipients, may exert different pharmacological or therapeutic effects (Wagner, 1971a). In other words, the therapeutic efficacy of a drug is not only a function of its intrinsic pharmacological and pharmacokinetic properties, but also of the physical form in which it is administered and of the various additives used as pharmaceutical formulation aids. Biopharmaceutics may be defined as the study of the influence of formulation on the therapeutic activity of a drug product. Recently, Wagner (1971b) gave a detailed definition of biopharmaceutics: "the study of the relationships among (1) the physical and chemical properties of the drug, (2) the physico-chemical and pharmaceutical properties of the dosage form, (3) physiological factors, (4) pharmacokinetic parameters, and (5) biological, pharmacological and clinical effects".

Several recent surveys of the field of biopharmaceutics have appeared (Levy, 1970; Gibaldi, 1971; Notari, 1971; Garrett, 1971; Wagner, 1971a), some of which have emphasized the principles underlying dosage form design (Wagner, 1971b; Benet, 1973). Often, evaluation of pharmacological and therapeutic responses in man in a quantitative manner is very complex. Because of the relationship between pharmacological effect and drug concentrations in the plasma, it is commonly accepted that concentration-time patterns of a drug in biological fluids can be used to compare the biological performance of drug products (Levy, 1968a; Ritschel, 1972; *Guidelines*, 1972). The determination of drug concentrations in the blood or plasma or drug amounts in the urine serves as a measure of the efficiency of a dosage form in delivering the drug to the general circulation. The relative amount to which an administered drug reaches the general circulation intact is defined as the bioavailability (Wagner, 1971a).

In the context of this definition, general circulation refers primarily to the venous blood (except the hepatic portal blood during the absorptive phase) and arterial blood, which carry the drug to the tissues. Often, the rate at which the drug reaches the general circulation also is included in the definition of bioavailability (*Guidelines,* 1972). However, this may sometimes be ambiguous (Wagner, 1973; Moss and Rhodes, 1974) and in this thesis the rate is referred to by the term "absorption rate". Both, the bioavailability and the absorption rate determine the biological performance or therapeutic efficacy of a drug product (Levy and Barr, 1972; Breimer, 1973). Biopharmaceutics mainly deals with the study of the factors influencing these two aspects of drug products. Also the route of administration should be considered relevant in this respect (Riegelman and Rowland, 1973). In this Chapter the kinetics of drug absorption and the estimation of bioavailability will be outlined. In addition, some important physiological and dosage form dependent factors influencing bioavailability and absorption rate will be discussed, mainly in relation to oral administration.

KINETICS OF DRUG ABSORPTION

When a drug is administered by any route of administration other than i.v. injection or infusion, many processes may be involved before it reaches the general circulation. Among these processes are the disintegration of the dosage form, dissolution of the active ingredient, transport to the absorption site and the actual drug absorption. These are time-dependent, simultaneously occurring processes, one of which determines the overall rate at which the drug reaches the general circulation (absorption rate). The kinetics of drug absorption may be assessed by measuring plasma concentrations at frequent intervals after drug administration. In spite of the complexity of the processes involved, the plasma concentration data, obtained from orally administered drugs, often appear to require only two exponential terms to describe the curve. If it is assumed that drug absorption and drug disposition can each be represented by a single first-order process, the following differential equations are valid:

$$\frac{dQ_1}{dt} = k_a C_o - k_{el} C_p \qquad (1)$$

$$\frac{dQ_{el}}{dt} = k_{el} C_p \qquad (2)$$

where Q_1 is the amount of drug in the body, viz. the amount (mg) of drug absorbed minus the amount of drug eliminated (Q_{el}); C_o is the concentration (mg/l) in the compartment from which absorption takes place (gastrointestinal

tract) and C_p is the plasma concentration; k_a is the absorption clearance constant (ml/min) and k_{el} the elimination clearance constant (ml/min). After integration of eq. (1), the following is obtained for the plasma concentration (van Rossum, 1971):

$$C_p = A \, . \, (e^{-t/\tau_{el}} - e^{-t/\tau_a}) \tag{3}$$

where τ_{el} and τ_a are the time constants (min) of elimination and absorption respectively, and A is the coefficient (mg/l). In Fig. 1 an example is given of a curve that can be described by eq. (3). If absorption proceeds much more rapid than elimination ($\tau_a \ll \tau_{el}$), then the elimination half-life of the drug can be deduced from the descending part of the curve ($t_{1/2el} = 0.69\ \tau_{el}$). The half-life of absorption ($t_{1/2a} = 0.69\ \tau_a$) can be obtained by the subtraction method or method of residuals. This method involves extrapolating the linear elimination phase of the semi-logarithmic plasma concentration-time plot to t = 0 and subtracting the plasma concentration data during the absorption phase from the corresponding concentration values on the extrapolated line. The absorption time constant can be determined from the straight line representing the absorption phase (Fig. 1). The coefficient A, also called the "zero-time concen-

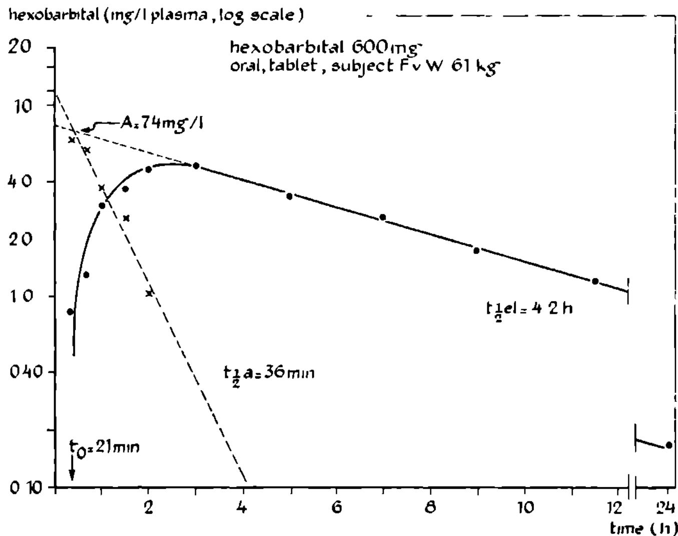


FIG. 1. Plasma concentration curve, on semi-logarithmic scale, following oral administration of 600 mg hexobarbital to a healthy volunteer. The dotted line, corresponding to a half-life of 36 min, represents the absorption phase. The latter was obtained by the subtraction method. The experimental plasma concentration curve can be described by the bi-exponential eq. (3).

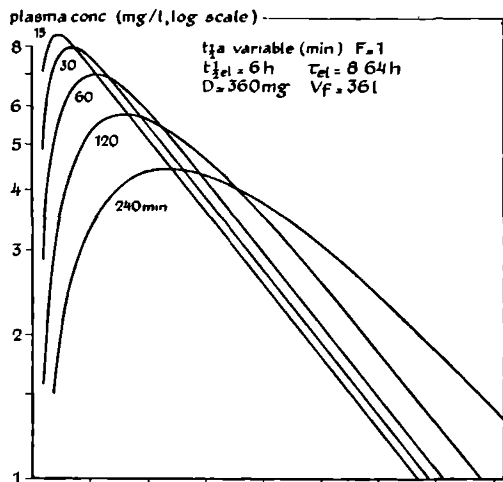
tration", is the concentration of the intersection of the elimination line and the absorption line. It may be derived that:

$$A = \frac{F.D}{V_f} \cdot \frac{\tau_{el}}{(\tau_{el} - \tau_a)} \quad (4)$$

where F is the bioavailability ($0 \leqslant F \leqslant 1$), D is the administered dose (mg) and V_f is the apparent volume of distribution (l). In practice crossing of the experimental lines often occurs some time after zero, which means that if first-order absorption is valid, then it lasts some time before absorption commences. This could be due, for example, to a slow disintegration of the dosage form or to a delay in the transit of the drug from the stomach to the small intestine. This "lag time" (t_0) has to be taken into account in eq. (3), where $t = t_{exp} - t_0$. A computer fit of the concentration data may give the best estimation of the model parameters, including the lag time (refer to vinylbital after oral and rectal administration, Chapter 4, Section III).

Other techniques, for the determination of the absorption time or rate constants, have been described by Wagner and Nelson (1963; 1964), who make use of percent absorbed-time plots. Loo and Riegelman (1968) have developed a procedure for the calculation of absorption rate constants for drugs which distribute according to a two-compartment open model. However, even if a two compartment model is apparent after analysis of i.v. data, the plasma curves after oral administration are frequently better fitted by a single-compartment model. If distribution of the drug into tissues occurs at a much faster rate than absorption, then drug disposition may be described by a single exponential process (compare Fig. 1 to Fig. 3 for hexobarbital). Special consideration has been given recently to the determination of absorption parameters for drugs with incomplete bioavailability (Notari et al., 1972; Perrier and Gibaldi, 1973; Leeson and Weintraub, 1973) and to the absorption kinetics of drugs released from prolonged action dosage forms (Ritschel, 1973).

In practice, it is often difficult to find the appropriate model that fits the concentration data during the absorption phase. Absorption may appear to be irregular, or too few data may be available to allow the estimation of the absorption parameters. An alternative method is the determination of the time after which the plasma concentration reaches its peak value (t_{max}). This peak time may be regarded as a rough measure of the absorption rate (Dittert and DiSanto, 1973). In Fig. 2 the influence of τ_a on the plasma concentration curve has been simulated. With increasing τ_a the maximum plasma concentration is reached at a later time and its height is lower. Furthermore, it is evident that, with slow absorption, samples should be collected for a long period of time in order to obtain the appropriate terminal elimination half-life.



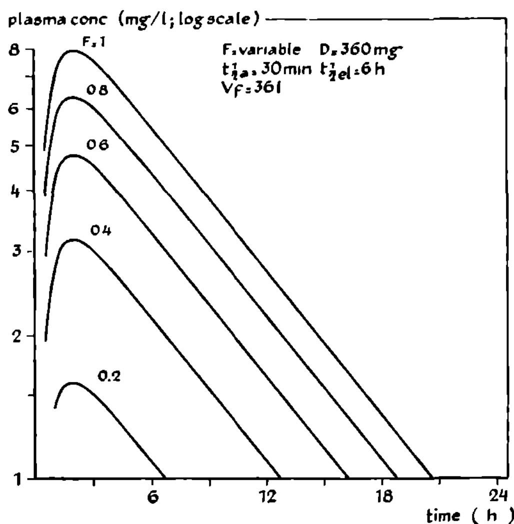


FIG. 2. Theoretical plasma concentration curves, on semi-logarithmic scale, based upon single-compartment kinetics and first-order absorption, following oral administration of the same drug with constant elimination pattern. The elimination phases are represented by a set of parallel straight lines. *Upper:* The decrease of the absorption rate (increase of the absorption half-life) results in a reduction of the maximum concentration and a lengthening of the peak level time. Bioavailability is assumed to be complete, which means that the AUC is the same in every case. *Lower:* The decrease of the bioavailability results in a proportional decrease of the maximum concentration as well as of the AUC.

According to the definition of bioavailability, a drug administered i.v. is completely available (F = 1 or 100%). Any other route of administration may result in incomplete bioavailability, due to dosage form factors or physiological factors. Bioavailability of a drug from a certain dosage form may be assessed by measuring the area under the plasma concentration curve, multiplied by the clearance constant (eq. (25) - (27), Chapter 1, Section I). In formula:

$$F = k_{el} \,.\, AUC/Dose \tag{5}$$

where k_{el} is the clearance constant (ml/min) and AUC the area under the curve from $t = 0$ to $t = \infty$ (mg.min/l). The clearance constant can only be estimated after i.v. administration; it is assumed that this parameter is dependent neither on dose nor time for a certain individual. For any route of administration other than i.v. only the quotient k_{el}/F can be obtained; the same holds for the apparent volume of distribution (V_f/F). A low bioavailability leads to an overestimation of these parameters. Measurements of the AUC can be made by using an appropriate numerical procedure, such as the trapezoidal rule or by determining the weight of the AUC relative to a standard area. An estimate of the AUC beyond the last concentration determined must be included. According to Dost's law of corresponding areas (Dost, 1968) the following applies:

$$AUC = \int_0^\infty C_p\,dt = \int_0^{t'} C_p\,dt + \int_{t'}^\infty C_p\,dt = AUC\Big/_0^{t'} + C_p^{t'} \,.\, \tau_{el} \tag{6}$$

where $C_p^{t'}$ is the last plasma concentration determined and τ_{el} is the terminal elimination time constant.

Absolute bioavailability of a drug from a dosage form can be determined by comparison with i.v. administration, preferably in the same individual. An example is shown in Fig. 3 for hexobarbital. If no i.v. data are available, bioavailability may be estimated relative to a standard dosage form:

$$F_{rel} = \frac{AUC_x \,.\, D_{st}}{AUC_{st} \,.\, D_x} \;(\times\ 100\%) \tag{7}$$

where the AUC_x and AUC_{st} are the infinite areas under the plasma concentration curves of the test dosage form (x) and the standard dosage form respectively. This method assumes that the plasma clearance is constant and that the

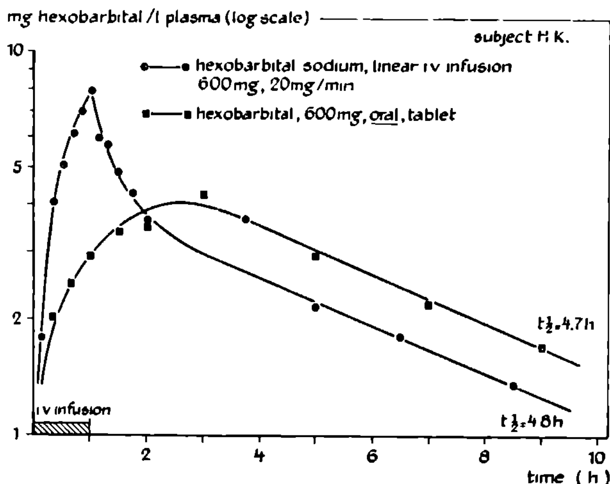


FIG. 3. Plasma concentration curves, on semi-logarithmic scale, following zero-order i.v. infusion of hexobarbital sodium and oral administration of hexobarbital to the same healthy volunteer. The elimination clearance constant was estimated from the i.v. data (two-compartment kinetics) and subsequently the bioavailability of the oral preparation was estimated (approximately 100%). Note the identical elimination half-lives after i.v. and oral administration, which suggests that the assumption of equal clearance at the two occasions may be correct.

AUC is proportional to the dose, viz. to F (Fig. 2). The best guarantee for the validity of these assumptions is the situation where the two preparations are administered in a cross-over manner to a panel of subjects (Wagner, 1971a). If two different panels of subjects are used a correction should be made for the difference in average body-weight if these are not practically the same (Ritschel, 1972). In the case of intra-individual variation of the half-life of a drug, which could be due to either a varying clearance constant or to a varying apparent volume of distribution, then bioavailability is difficult to assess exactly (refer to vinylbital, Chapter 4, Section III). Wagner (1967) has proposed that a "modified AUC" should be utilized in such situations, which corrects for the different half-lives. Other methods of bioavailability estimation include cumulative urinary excretion measurements or area measurements after multiple dosing (*Guidelines*, 1972). Van Rossum (1971) has proposed an elegant method for the assessment of bioavailability during chronic medication. For a given subject the plateau level is proportional to the bioavailability, provided that the elimination clearance constant remains constant.

The absorption of an orally or rectally administered drug from the gastrointestinal tract involves disintegration of the dosage form, dissolution of the drug into gastrointestinal fluids, passage to the site of absorption and transfer across the mucosal barrier into the portal circulation. In this sequence, the dissolution process is frequently the slowest process (Levy, 1968b). The dissolution rate is proportional to the surface area of the dissolving solid (Nernst and Brunner, 1904), and is therefore favoured by a rapid disintegration of the dosage form into fine particles. Absorption of slightly soluble drugs has been shown to be inversely proportional to particle size, e.g. griseofulvin (Atkinson et al., 1962; Fincher, 1968). Another variable is the crystal form of the drug particle since polymorphic forms may have different solubilities (Haleblian and McCrone, 1969). Many drugs, including barbiturates, have several polymorphic modifications (Brandstätter-Kuhnert, 1969), which generally differ in their dissolution rates. Since ionized molecules are more soluble in aqueous media than non-ionized molecules, the dissolution rate may be very dependent on the pK_a-value of a drug and the pH of the gastrointestinal fluid. Basic drugs are more soluble in the stomach than in the intestine and acidic drugs are less soluble in the stomach when compared to the higher pH of the intestinal fluids. Administration of barbiturates in the free acid form generally results in slow absorption, due to poor dissolution in the acid medium of the stomach (Section III). The most effective means of attaining higher dissolution rates is to use a highly water-soluble salt of the weak acid. The salt acts as its own buffer and raises the pH of an acidic dissolution medium in the immediate environment, surrounding the dissolving drug solids. The dissolution rate of the sodium salt of a weak acid may be many-fold higher than the dissolution rate of the free acid itself, even though the final equilibrium solubility of the drug and its salt are the same (Higuchi et al., 1958; Nelson, 1958). If the sodium salt is subsequently precipitated as the free acid in the bulk phase of an acidic medium, it will usually do so in the form of very fine particles, which have the proper characteristics for rapid redissolution. In this connection refer to the rapid absorption of hexobarbital and heptabarbital, when administered as their sodium salts (Chapter 1 and 5, Section III).

Many excipients used in the preparation of solid dosage forms (disintegrants, lubricants, fillers, wetting agents, etc.) can influence bioavailability and absorption rate (Levy, 1970). Complex formation and micelle formation are important processes that may interfere with drug dissolution, either by increasing or by decreasing the dissolution rate (Levy, 1970; Gibaldi and Feldman, 1970). Also

changes in drug manufacturing processes sometimes lead to variations in the biological performance of a drug product, e.g. digoxin (Lindenbaum et al., 1971). The critical factors for the different dosage forms have recently been reviewed by Polderman and Weekers-Andersen (1973). Special attention has been attributed to dosage forms that are intended for rectal application (Bevernage and Polderman, 1973).

It should be emphasized that bioavailability studies in vivo should be preceded by careful pharmaceutical analysis of the drug products to be investigated (quality control). This includes tests for identity, assay for active constituents, stability control and sometimes in vitro disintegration or dissolution tests (Pernarowski, 1971; Blake, 1971; Breimer, 1973). If, for instance, a dosage form does not contain the stated amount of active ingredient, the in vivo study will be useless beforehand. An in vitro dissolution test is only meaningful if the differences in dissolution rate can be correlated with measured differences in bioavailability following administration of the dosage form to human subjects (Benet, 1973). In future much effort has to be directed into this area of biopharmaceutics in order to find such correlations. Satisfactory results have been obtained already for digoxin (Dunning et al., 1973). In practice most bioavailability problems arise with drugs which exhibit a poor aqueous solubility (Riegelman, 1969). In this respect very limited information is available at present on hypnotic drugs.

PHYSIOLOGICAL FACTORS AFFECTING BIOAVAILABILITY AND ABSORPTION RATE

The transport of drugs across the gastrointestinal membranes is often a simple diffusion process and depends on concentration gradients, diffusion properties, membrane area and partition between membrane and solvent (Turner et al., 1970). For weak acids and bases partition is determined by their pK_a-values, the pH of the medium surrounding the membrane and the lipophility of unionized drug (Schanker, 1971). For barbiturates an excellent correlation has been found between the chloroform-water partition coefficient of the unionized form and the degree of in vitro absorption by the rat colon (Schanker, 1959). There is good evidence that the unionized form of a drug will be absorbed faster than the ionized form at any particular site in the gastrointestinal tract. However, it has recently been emphasized, that the rate of absorption of a drug from the intestine will be greater than the gastric absorption rate, even if the drug is ionized in the intestine and unionized in the stomach (Benet, 1973). The membrane surface area in the intestine is several fold greater than that found in the

stomach (Gordon and Bruckner-Kardoss, 1966), which favours rapid absorption. For example, Doluisio et al. (1969) showed that the absorption rate for the weakly acidic drugs salicylic acid and barbital from the intestine of rats (pH 6.0) was 5 to 12 times higher than the gastric absorption rates at pH 3.0. In humans the absorption of warfarin and acetylsalicylic acid from the intestine was found to be two or three times faster than from the stomach (Siurala et al., 1969; Kekki et al., 1971). If the intestine is the major site of absorption of most drugs, then it may be expected that stomach emptying is a rate determining step for drug absorption. Recent studies in man have shown correlations between stomach emptying and absorption rate of, e.g. digitoxin (Beermann et al., 1971), L-Dopa (Bianchine et al., 1971) and paracetamol (Heading et al., 1973). There are many factors which could affect the stomach emptying rate and thereby retard absorption, including food, volume and viscosity of stomach contents, pH, osmotic pressure, age, health and position of the subject (Davenport, 1971). Food was shown to decrease the absorption rate of pentobarbital sodium in healthy subjects, but not to affect the bioavailability (Smith et al., 1973). Alternatively, large volumes of liquids with low viscosity (water) may enhance the rate of absorption by increasing the initial rate of drug transfer to the small intestine (Levy, 1970). Bed rest may be another contributing factor, since Barr et al. (1972) showed that tetracycline absorption diminished in bedridden patients when compared with ambulatory subjects. Therefore, Riegelman and Rowland (1973) have emphasized to examine the bioavailability of drugs in conditions simulating clinically relevant circumstances. In the present investigations with hypnotic drugs, the volunteers were requested to remain in an upright position for about 15 min after drug administration and then to lie down for a few hours. This procedure was chosen in order to simulate as closely as possible the taking of a hypnotic drug in practice.

One of the most important factors affecting bioavailability after oral administration is drug metabolism. When a drug is absorbed from the gastrointestinal tract it must first pass through the liver before reaching the general circulation. If metabolism occurs in the liver, part of the drug will be inactivated during first passage through the liver. If the metabolizing capacity of the liver is great for a certain drug, then this "first-pass" effect on the bioavailability may be substantial. The area under the plasma concentration curve after oral administration may be considerably less than the corresponding area following i.v. administration. (Harris and Riegelman, 1969; Gibaldi et al., 1971; Gibaldi and Feldman, 1972). Rowland (1972) has shown that the unmetabolized fraction (Θ) of an oral dose appearing in the general blood circulation may be given by:

$$\Theta = 1 - \frac{\text{hepatic clearance}}{\text{liver blood flow}} \tag{8}$$

Thus, if the hepatic clearance for a drug is large compared to liver blood flow (about 1500 ml/min), a substantial "first-pass" effect may be expected. For example, the bioavailability of lidocaine (Boyes et al., 1971) and propranolol (Shand and Rangno, 1972) after oral administration is significantly reduced compared to i.v. administration. A similar phenomenon was observed for methohexital (Chapter 3, Section III). For every drug with a high metabolic clearance a "first-pass" effect should be considered when given by a route which initially leads to the liver after drug absorption. It has been stated frequently that rectal administration would result in the bypass of the liver. There is, however, no convincing evidence for this supposition and Schwarz (1966) has criticized this assumption on physiological grounds.

In addition to liver metabolism, enzymes within the gastrointestinal fluids, microflora and mucosa are capable of drug metabolism to a greater or lesser extent (Sheline, 1968; Aito, 1973). For instance salicylamide (Barr and Riegelman, 1970) and isoprenaline (Dollery et al., 1971) are glucuronidated and sulfated respectively in the gut wall, which results in poor bioavailability.

Riegelman and Rowland (1973) have discussed recently the influence of saturation phenomena on the bioavailability of drugs which are subject to substantial metabolism in the gastrointestinal tract or the liver. The extent of the dose and the rate at which the drug is presented to the metabolizing enzymes appear to be critical factors.

When a drug product is found to exhibit poor bioavailability, it is important to distinguish between dosage form and physiologically modified bioavailability. If pharmaceutical factors are involved, then suitable changes in the formulation or manufacturing processes may often lead to improvement. Unfortunately, physiological factors are more difficult to control, except by parenteral administration. The choice of another drug substance with comparable pharmacological activity, but with more appropriate absorption and metabolism characteristics may be the best solution for such a case (Ariëns, 1971; Riegelman and Rowland, 1973).

EFFECT OF BIOAVAILABILITY AND ABSORPTION RATE ON DRUG ACTION

As stated earlier, the bioavailability and the absorption rate of a drug from a drug product will determine the therapeutic efficacy of the product. Incomplete bioavailability of a drug from a certain dosage form is basically equivalent to a reduction in the administered dose. This results in decreased drug levels in

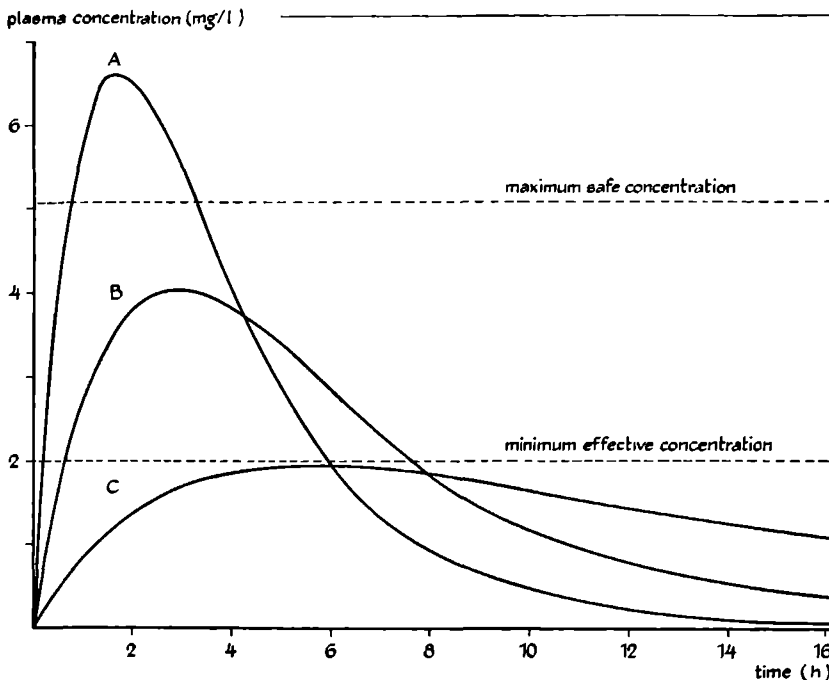


FIG. 4. Theoretical plasma level profiles illustrating how changes in absorption rate may influence the onset, intensity and duration of drug action. The size of the dose and the bioavailability of the dosage forms A, B and C are the same. *A*: Due to the very rapid absorption the concentration exceeds the maximum safe concentration. However, by decreasing the dose this could be a suitable preparation if a very rapid onset of action is required. *B*: This profile may be regarded as normal after oral administration. *C*: Due to the slow absorption there will be no effect. However, by increasing the dose this could be a suitable preparation with prolonged action.

the body (Fig. 2) and in decreased pharmacological effects. Wagner (1971a) and Prescott and Nimmo (1971) have reviewed a number of examples concerning incomplete bioavailability of drug products. The great variation of the bioavailability of digoxin from various tablets is another example, which is of great clinical importance (Lindenbaum et al., 1971; Dunning et al., 1973; van Zwieten, 1973). Often, only the absorption rate of a drug varies from one dosage form to the other. In Fig. 4 the hypothetical plasma concentration profile of three oral preparations is presented, each containing the same amount of active drug and exhibiting complete bioavailability. Absorption, however, occurs at different rates. The onset of therapeutic activity is assumed to occur at the

time the drug concentration reaches the minimum effective concentration, whereas toxic effects may become apparent above the maximum safe concentration. It is evident that dosage form A would be the suitable choice when rapid onset of action is desired. However, the dose of this formulation should be diminished in order to avoid adverse reactions. Absorption from dosage form B is slightly slower than from A, however, there is less risk of toxic effects with this preparation. Dosage form C is formulated in such a way, that no therapeutic action can be expected. However, by increasing the dose, this formulation could be beneficially used as a prolonged action product. The absorption profile of a drug product should be known in order to allow the judgement of its usefulness for a certain therapy. Firstly, the desired onset and

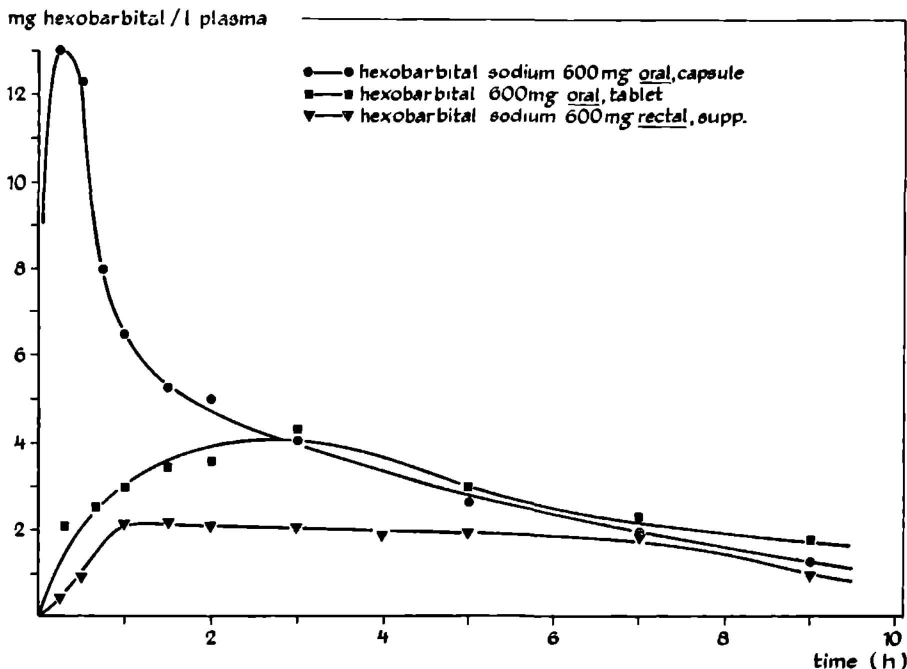


FIG. 5. Plasma concentration curves following oral and rectal administration of hexobarbital or hexobarbital sodium to a healthy volunteer. Note the ressemblance of these plasma level profiles with the theoretical ones in Fig. 4. Hexobarbital sodium, when administered orally, may be used for the rapid induction of sleep. Possibly a lower dose would be sufficient to achieve this purpose. If the maintenance of sleep is the primary prerequisite of hypnotic drug treatment, then hexobarbital free acid could be used. Administration of the suppository results in a rather constant plasma level for several hours. This may be beneficial in instances where a constant degree of sedation is required.

duration of therapeutic action should be defined and secondly a proper choice of drug and dosage form can be made. Fig. 5 may be regarded as a practical illustration of the various possibilities indicated in Fig. 4. Hexobarbital, when administered as the sodium salt, is very rapidly absorbed which is favourable for rapid sleep induction. Absorption of hexobarbital after administration of the free acid is much slower. However, if sleep maintenance is a prerequisite of hypnotic drug treatment, then the acid form could be used. Finally, the suppository yields a relatively low plasma level, which remains constant for several hours. This preparation may be considered a judicious choice for sedative purposes, since the intensity of effect should be constant for longer time periods in sedative drug treatment. It is evident that the same active drug, incorporated in different pharmaceutical formulations or applied in a different chemical form, may positively be used for different therapeutic objectives. A prerequisite is knowledge pertaining to the plasma level profile of the various preparations.

Often, plateau or steady state drug levels are achieved during chronic drug administration. These are usually independent of absorption rate, but the height of the plateau level is directly proportional to the bioavailability of a drug product (van Rossum, 1971). Only if the dosing interval is greater than the apparent biological half-life will there be significant fluctuations in drug levels that could be modified by changing the absorption rate of a drug. It should be emphasized here that the importance of differences between drug products, with respect to bioavailability or absorption rate, depends primarily upon its intended therapeutic use. For a short-acting hypnotic drug, for example, a relatively small difference in the rate of absorption may have significant influence on the onset of action, which in turn may affect its therapeutic usage; whereas a similar difference in bioavailability may be of no importance. However, for a chronically administered drug a relatively large difference in rate of absorption may have a minimal effect on its therapeutic usage, whereas a small difference in bioavailability may become highly significant, either by reducing the average steady state concentration below the effective level, or by increasing the concentration into the range where adverse reactions become apparent.

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CHAPTER 3

HYPNOTIC DRUG THERAPY PHARMACOKINETIC AND BIOPHARMACEUTICAL ASPECTS

INTRODUCTION

Hypnotic drugs may induce sleep when given at night or cause sedation when given during day-time. They are widely used for the treatment of insomnia, which of all sleep disturbances is the one most frequently encountered in general practice (Kales and Kales, 1974). However, insomnia cannot be regarded as an illness in itself, but as a symptom of an underlying disorder (physical or emotional), which remains to be diagnosed and treated (Pollitt, 1973). Nevertheless, it may be necessary to prescribe a hypnotic drug for the temporary relief of these symptoms, until the underlying cause will be adequately dealt with.

The effectiveness of hypnotic drugs in inducing and maintaining sleep is generally determined by clinical evaluation. This is assessed with regard to the subjective impressions expressed by patients, nursing staff and physicians (for review: Zelvelder, 1971). More objective measures have been developed recently in the so-called sleep laboratories (Kales and Kales, 1970; Kales et al., 1970) which are mainly concerned with assessment of drug effects on the whole night's sleep, during several consecutive nights. Sleep is divided into rapid eye movement (REM) and non-rapid eye movement (NREM) stages. During REM sleep there are intermittent bursts of eye movement, a marked decrease of muscle tone and a low amplitude, fast frequency electroencephalogram. In NREM sleep there is a progressive slowing of the frequency and increase in amplitude of electroencephalogram waves (Jouvet, 1967; Kales, 1969). These various stages occur intermittently during normal sleep and sleep disturbances may be characterized by deviations from the normal pattern (Kales and Kales, 1974). Psychoactive agents, including hypnotic drugs, have been shown to affect the relative proportion and duration of REM sleep or other stages, but the significance of these findings in relation to insomnia and the choice

and use of hypnotic drugs is not yet clear (Hartmann, 1968; Oswald, 1968; Kales et al., 1970).

Important requirements which a hypnotic drug should fulfil, in addition to satisfactory pharmacodynamic properties in terms of facilitating sleep induction and maintaining sleep, concern its onset and duration of action. Since most hypnotic drugs are taken by ambulatory people, the central depressant effect of the drug should have declined sufficiently to be unimportant, both subjectively and objectively, the morning following the night of drug intake. The ability to carry out various skilled tasks, including the driving of a motor-vehicle, should be unimpaired. Inactivation of most hypnotic drugs occurs by metabolism in the liver, which results in a decrease of the plasma concentration and a concomitant withdrawal of the drug from its site of action in the central nervous system. The kinetics of drug distribution and drug elimination are pertinent to the duration of action, where the plasma half-life is regarded as a measure for the rate of drug inactivation (Chapter 1, Section I). With respect to the onset of action the rate of drug absorption, which mainly depends on biopharmaceutical factors, is of great importance.

PHARMACOKINETIC ASPECTS OF HYPNOTIC DRUG THERAPY

It is generally appreciated that a distinct limitation to the duration of drug

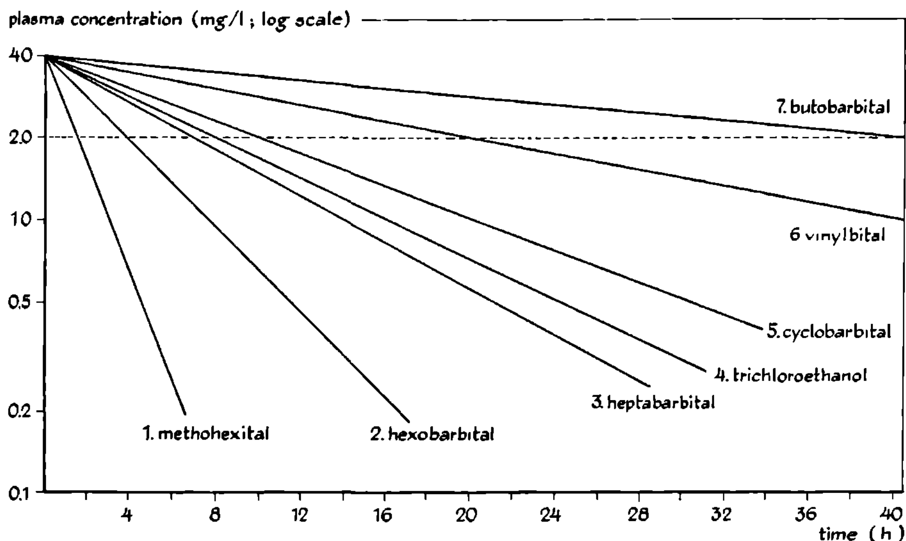

FIG. 1. Average plasma level profile of the compounds which were investigated (semi-logarithmic scale). For half-lives see Table I.

action is an important aspect of the treatment of insomnia. Therefore, it is remarkable that the pharmacokinetics of relatively few hypnotic drugs have been well defined. The historical classification of the barbiturates, for example, into long-, intermediate-, short- and ultrashort-acting categories is based upon the duration of action when injected i.v. into rabbits (Tatum, 1939). Mark (1969) has seriously questioned this classification by arguing that every barbiturate, in principle, may be described as long-acting or short-acting, dependent on the administered dose. The duration of drug action is determined by the dose applied and the rate of inactivation, assuming a constant minimal effective concentration. A special situation is encountered with the barbiturates used for i.v. anaesthesia, where the redistribution of drug from the central nervous system to other parts of the body is primarily responsible for the termination of the anaesthetic effect (Price et al., 1960). However, in this case the rate of elimination will also determine the duration of the residual depressant action. For many barbiturates the half-life of elimination is not known after administration of therapeutic doses to man. Martindale's Extra Pharmacopoeia (1972) erroneously suggests that the half-lives are similar for those compounds classified as having the same duration of action.

In Fig. 1 and 2 the average plasma levels of the drugs described in this thesis are shown. These compounds are all used for the same purpose (with a possible exception of methohexital), viz. for the treatment of insomnia. Hexo-

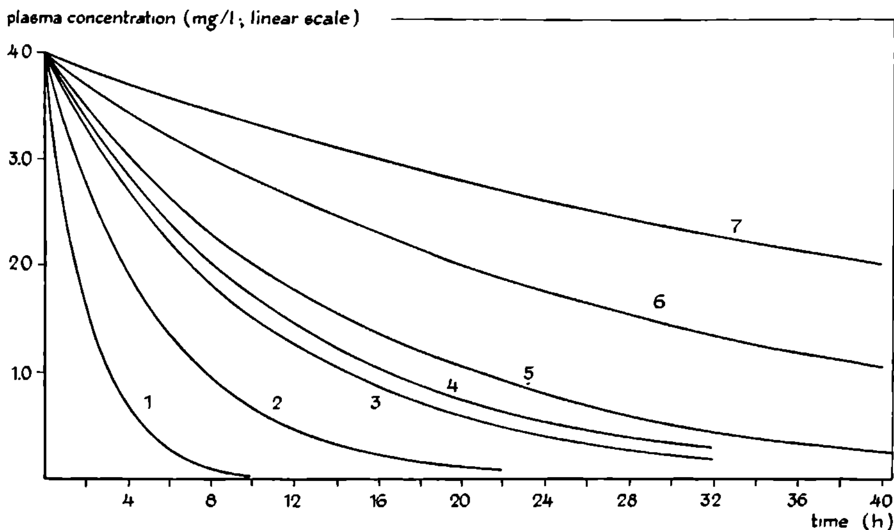

FIG. 2. Average plasma level profile of the same compounds as in Fig. 1 on linear scale.

barbital, heptabarbital, cyclobarbital, vinylbital and butobarbital have been classified as intermediate-acting barbiturates (Martindale, 1972). Their average half-life varies from 4.4 h for hexobarbital to 37.5 h for butobarbital. Obviously, considerable differences in duration of action between these compounds may be expected, which may only partly be corrected for by adjusting the administered dose. In Table I the half-lives of some of the drugs used for the treatment of insomnia are listed. For many drugs in common use the disappearance rate from the body is fairly slow with respect to their intended duration of action (8 - 10 h). Also, the formation of active metabolites should be considered. For instance, flurazepam can only be detected for a short period of time in the plasma, but its N-desalkyl metabolite, which has comparable pharmacological properties (Randall and Kappell, 1973), possesses a half-life of 47 - 100 h (Kaplan et al., 1973). After oral administration of chloral hydrate, its metabolite trichloroethanol causes the central depressant activity (Marshall and Owens, 1954). Metabolites of barbiturates are usually not pharmacologically active.

Due to the long half-life of many hypnotic drugs, central nervous system depressant effects are likely to persist into the following day. This may give rise to feelings of „hang-over" and drowsiness and furthermore to an impaired ability to perform daily tasks. For many hypnotic drugs these effects have been described, although in few clinical trials on the effectiveness of hypnotic drugs, this aspect has been evaluated in an objective manner. Only recently has evaluation thereof become part of sleep laboratory studies (Bixler et al., 1973). Malpas et al. (1970) have studied the residual effects of nitrazepam (5 and 10 mg), amobarbital (100 and 200 mg) and placebo. Although all volunteers who participated in this study reported a good night's sleep and considered themselves to be alert on awakening, they were unaware of significant impairment of performance 13 h after all drug treatments. Similarly, performance of simple tasks was impaired at least up to 8 h after a single oral dose of 100 mg pentobarbital (Goodnow et al., 1951; Von Felsinger et al., 1953) and 100 mg secobarbital (Kornetsky et al., 1959; Bixler et al., 1973), whereas 200 mg secobarbital caused impairment at least up to 22 h (McKenzie and Elliott, 1965). Residual effects on both psychological and physiological functions have been found up to 18 h after butobarbital and nitrazepam administration (Bond and Lader, 1972). These authors also found, that the subjects who slept inadequately after intake of a placebo, did not exhibit impairment of performance the following day. This suggests that incidental lack of sleep does not necessarily lead to comparable after-effects as encountered with hypnotic drugs. The cited literature data on the residual effects of hypnotic drugs are

in agreement with the long half-lives of the drugs investigated (Table I). Also, residual effects were shown to exist the following day for flurazepam (Bond and Lader, 1973; Bixler et al., 1973), which is consistent with the long half-life of the active N-desalkyl metabolite.

Another consequence of the persistence of hypnotic drugs in the body may be the exaggerated central depressant effect, when alcoholic beverages are taken even a long time after intake of the drug. This may give rise to dangerous situations. Doenicke (1962) studied the influence of a single dose of 200 mg butabarbital combined with alcohol in relation to traffic safety. He concluded that abstention from alcohol is essential for up to 24 h after barbiturate administration, since even small quantities of alcohol, administered at various times after butabarbital, caused severe impairment of mental and physical performance.

Frequently, hypnotic drugs are taken every night. Substantial accumulation of a drug will occur if its half-life is longer than a dosage interval of 24 h. For phenobarbital, when used in anti-epileptic therapy, this is of advantage since a constant steady state level is required for such therapy. For the treatment of insomnia the accumulation of a certain compound is definitely undesirable. Substantial accumulation was shown to occur in the case of butobarbital (Chapter 6, Section III), with a half-life of 34 - 42 h, when administered for several consecutive nights. The accumulation is counteracted to a certain degree by the development of microsomal enzyme induction, which results in a shortening of the half-life. Often, this also causes an enhanced rate of metabolism of other, concomitantly administered drugs, which should be reckoned with. Accumulation of a hypnotic drug in the body does not necessarily imply that there is a concomitant intensification of central depressant effects, since pharmacodynamic tolerance is likely to develop (Sharpless, 1970). It should further be considered that prolonged use of hypnotic drugs often leads to drug dependence (Jaffe, 1970). The marked sleep and dream alterations associated with drug withdrawal are primary factors in the development of hypnotic drug dependency (Oswald and Priest, 1965; Kales and Kales, 1970). To what extent pharmacokinetic factors are involved in this phenomenon is uncertain, although it may be speculated that the continuous exposure of the central nervous system to relatively high drug concentrations may be a factor for consideration.

It will be clear that many of the undesired effects of hypnotic drugs may be attributed to the persistence of the drug in the body. Therefore, it seems more suitable to use drugs with a relatively short half-life. Arbitrarily, one could require that the half-life of a hypnotic drug should not exceed the duration of a night's sleep (8 - 10 h). During such a time period at least 50% of the drug

TABLE I. Plasma or blood half-lives of drugs used for the treatment of insomnia (mean values in parentheses).

Drug	Half-life (h)	Reference
Barbiturates and related compounds [1])		
allobarbital	30 - 50	Lous, 1954
amobarbital (Amytal®)	20 - 25 (22.7) 14 - 42 (24.8)	Balasubramaniam et al., 1970 Kadar et al., 1973
barbital	4 - 5 days	Wilbrandt, 1964
butobarbital (Soneryl®)	34 - 42 (37.5)	This thesis; chapter 6, Section III
cyclobarbital (Phanodorm®)	8 - 17 (11.6)	This thesis; chapter 7, Section III
glutethimide (Doriden®)	5 - 22 (11.6)	Curry et al., 1971
heptabarbital (Medomin®)	6 - 11 (7.6)	This thesis; chapter 5, Section III
hexobarbital (Evipan®)	3 - 7 (4.4)	This thesis; chapter 1, Section III
methohexital (Brietal®)	0.7 - 1.0 (oral) 1.2 - 2.1 (i.v.)	This thesis; chapter 3, Section III
pentobarbital (Nembutal®)	23 - 30 (26.3)	Smith et al., 1973
phenobarbital	2 - 6 days	Maynert, 1972
secobarbital (Seconal®)	28	Fazekas et al., 1956
vinylbital (Bykonox®)	18 - 34 (23.7)	This thesis; chapter 4, Section III

thiopental (Pentothal®)	>8	Brodie, 1952
Benzodiazepines		
nitrazepam (Mogadon®)	21 - 28 (25.1)	Rieder, 1973
flurazepam (Dalmadorm®)	not measurable	
1-desalkyl-flurazepam	47 - 100 (65.5)	Kaplan et al., 1973
diazepam (Valium®; Levium®)	20 - 42 (28)	
desmethyl-diazepam	42 - 96 (57)	van der Kleijn et al., 1971
Miscellaneous		
bromide	7.5 days	Wilbrandt, 1964
chloral hydrate	very short; not measurable after oral administration	
trichloroethanol	7 - 10 (8.0)	This thesis; chapter 8, Section III
meprobamate	6 - 17	Hollister and Levy, 1964
	11 - 15 (12.5)	Held and von Oldershausen, 1969
methaqualone[2]	20 - 42 (32.6)	Alvàn et al., 1973

[1]) For reviews of the metabolism of barbiturates see Mark (1963) and Parke (1971).

[2]) Morris et al. (1972) reported a half-life of 2.6 h for methaqualone. However, these authors measured plasma levels up to 8 h after drug administration and determined in fact only the half-life of the distribution phase. Alvàn et al. (1973) measured plasma concentrations up to 130 h after a single dose by using mass fragmentography.

will have been inactivated, which may be sufficient to decrease its concentration in the brain below the apparent minimal effective central depressant concentration. It should be realized, however, that this is very dependent on the dose administered and it may vary substantially from one person to the other.

BIOPHARMACEUTICAL ASPECTS OF HYPNOTIC DRUG THERAPY

Patients who suffer from insomnia have been divided into at least three categories (Karacan and Williams, 1971): 1. those who experience difficulty in getting to sleep; 2. those who experience intermittent or lacunary sleep, with one or more periods of wakefulness in the middle of the night; 3. those who experience early morning awakening. It is commonly recommended that the first category of patients are treated with a „short-acting" hypnotic drug and the other two categories with an „intermediate-" or „long-acting" drug. Inadequate attention has been paid to the biopharmaceutical aspects of hypnotic drug therapy, in spite of the fact that these may offer a more rational approach to the treatment of the various categories of insomniacs. A prerequisite for adequate treatment of the patients who have difficulty in getting to sleep is that the drug should be rapidly absorbed, in order to be effective within 30 min after retiring. When early sleep is not obtained, the patient may be tempted to take a second dose, which may lead to overdosage and prolonged effects. Thus, such a dosage form should be used from which the drug is known to be rapidly absorbed. This is the more important, since most patients who take a hypnotic drug at night, have not been fasting for a few hours prior to retiring. Food may delay the absorption of a hypnotic drug as has been shown for pentobarbital (Smith et al., 1973) and for capuride (Johnson et al., 1973). The half-life of a drug which is used for the treatment of difficulties getting to sleep should be short, since the required duration of effect is relatively short and natural sleep should predominate again as soon as possible once sleep has been induced.

For the second and third category of insomniacs, rapid absorption is not primarily required. Even delayed absorption may be desirable if a drug with a short half-life is used, such that at a later time an effective concentration is reached. This is more preferable than a drug with a longer half-life, since the latter brings along a higher risk of residual effects during the following day. Firstly, the desired onset and duration of hypnotic or sedative action should be defined and then a proper choice of drug and dosage form can be made. By applying the principles of biopharmaceutics underlying dosage form design, the same drug incorporated in different pharmaceutical formulations may serve

different therapeutic aims (refer to Chapter 2, Section I). This seems to be a rational approach with regard to hypnotic drug therapy.

THE INVESTIGATIONS DESCRIBED IN THIS THESIS

The primary aim of the investigations described in this thesis was to study the pharmacokinetics of hypnotic drugs in man, in order to obtain important information concerning their utility in general practice. Barbiturates were studied, since they are still an important class of hypnotic drugs. Their clinical effectiveness has been well defined, whereas pharmacokinetic information of many barbiturates after administration of therapeutic doses to man is lacking. Further, it was considered of interest from a more fundamental point of view, to study the kinetic behaviour of structurally closely related compounds. Alvarez (1971) and Yih (1974) found considerable differences in elimination half-lives for the various barbiturates in animals. Based upon their findings a number of compounds with varying half-lives was chosen for the study in man, where the major attention was paid to the compounds with relatively short half-lives, since these seemed most rational for hypnotic drug therapy. Chloral hydrate is still a widely used hypnotic drug and was therefore included in these studies.

For a study on human pharmacokinetics the primary prerequisite is the availability of a method for the quantitative determination of drug in plasma. The method should be specific and sensitive, in order to allow concentration measurements for relatively long time periods after administration of a single therapeutic dose to man. For barbiturates such a method has been developed by using gas chromatography with nitrogen selective detection (Chapter 1 to 8, Section II). For chloral hydrate and metabolites head-space analysis with electron capture gas chromatography was employed (Chapter 9, Section II).

Usually single dose experiments were performed in healthy male volunteers in order to assess the main pharmacokinetic characteristics of a drug. Additional information was obtained by administering the drug repetitively to a certain individual. Accumulation and the development of enzyme induction, particularly with barbiturates may become apparent after repetitive drug administration. Although the central depressant effects were not objectively assessed in these investigations, it was tried to obtain some information on this matter by interrogation of the volunteers at regular times about feelings of drowsiness and sleepiness. The conclusions drawn at the end of each Chapter of Section III as to whether a certain drug should be used in general practice are solely

based upon its pharmacokinetic behaviour, though. Obviously, the ultimate choice of a drug to be used in actual therapy depends upon many other factors as well, including the pharmacodynamic and toxicological properties of the drug and the particular complaints and condition of the patient.

In addition to the pharmacokinetic aspects of the various hypnotic drugs were the biopharmaceutical aspects studied. Several dosage forms were investigated in order to assess their ability to deliver the active ingredient with the appropriate rate and in sufficient amount to the general circulation. Already existing drug products were included in the studies and new dosage forms were developed, according to the absorption rate required.

With hexobarbital also more fundamental aspects have been evaluated. This barbiturate possesses an asymmetric centre and it was considered of interest to investigate the kinetics of elimination of the separate enantiomers, both in man and in rats (Chapter 2, Section III). Furthermore, in cooperation with the Medizinische Universitätsklinik at Würzburg, GFR, the kinetics of hexobarbital were studied in patients with liver disease in order to obtain information concerning possibly impaired drug metabolism and other kinetic changes associated with pathological conditions (Chapter 1, Section IV).

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SECTION II

ANALYTICAL PROCEDURE

THE GAS CHROMATOGRAPHIC DETERMINATION OF BARBITURATES IN PLASMA

INTRODUCTION

Two important methods for the analysis of barbiturates in plasma are available; ultraviolet spectrophotometry and gas chromatography. The U.V. - method implies the extraction of the compounds from plasma, usually with chloroform, and then re-extraction takes place from the organic phase with strong alkali. Quantitation is based on the absorbance difference between buffer pH 10 and the barbiturate solution. However, this procedure does not distinguish between different barbiturates or barbiturate metabolites with intact pyrimidine ring and is therefore unspecific. Gas chromatography on the other hand is primarily a separation technique, which offers the advantage of far greater specificity. In addition a greater accuracy and sensitivity can be achieved, which makes gas chromatography the method of choice for the determination of barbiturates in biological fluids (Hathway, 1971).

Since the first attempt to employ gas chromatography in the identification of barbiturates was made by Janak (1960), several reports on the qualitative and quantitative analysis of barbiturates in biological fluids have appeared. McMartin and Street (1966) have reviewed the literature up to 1965, which mainly dealt with column technology in relation to a good separation and resolution of mixtures of barbiturates. Since then several methods for the quantitative determination in plasma or blood and urine have been described. However, these lack sensitivity for measuring therapeutic levels in man (Anders, 1966; Martin and Driscoll, 1966; Leach and Toseland, 1968; Bloomer et al., 1970; Sine et al., 1970; MacGee, 1971; Street, 1971; Fiereck and Tietz, 1971; Cooper et al., 1972; Berry, 1973). All of these procedures are suitable for the determination of overdose levels, which are in general higher than 10 mg/l plasma (Winek, 1970; Hathway, 1971). Therapeutic levels, on the other hand are lower and particularly if it is intended to study the kinetics of a drug, it is necessary to follow the plasma concentration for longer periods of time until

very low concentrations have been reached (van Rossum et al., 1971). An exception is phenobarbital, which is used as an antiepileptic agent, the steady state levels of which are much higher than those encountered in sedative and hypnotic drug therapy (Kupferberg, 1970; Baylis et al., 1970; Meijer, 1971; Berlin et al., 1972; Gardner-Thorpe et al., 1972). Sensitive methods are available for amobarbital and pentobarbital (Inaba and Kalow, 1972), but these are sometimes laborious (Balasubramanian et al., 1969; Smith et al., 1973) or they require the formation of derivatives (Ehrnebo et al., 1972). Williams et al., (1973) obtained a higher sensitivity by adding formic acid to the carrier gas.

The application of combined gas chromatography - mass spectrometry for the determination of barbiturates in plasma was described by Draffan et al., (1973). This technique is particularly suitable when only small plasma samples can be obtained (e.g. in infants and children). The same holds for the radioimmunoassay technique that has been developed for barbiturates (Spector and Flynn, 1971; Flynn and Spector, 1972).

In all gas chromatographic procedures described hitherto, use has been made of the normal flame ionization detector. As already mentioned, sensitivity and probably also selectivity is unsatisfactory for a rapid determination of therapeutic levels of barbiturates in man. Therefore the nitrogen selective flame ionization detector was selected, which seems to offer new possibilities for the sensitive determination of nitrogen containing drugs in biological fluids (Donike et al., 1970). Goudie and Burnett (1973) have described the simultaneous determination of phenobarbital, primidone and phenytoin in serum using a nitrogen detector. Other nitrogen containing drugs such as amphetamines and narcotic analgesics have been analyzed in this way (Riedmann, 1972a; James and Waring, 1973).

THE NITROGEN DETECTOR

The nitrogen flame ionization detector - also called alkali flame ionization detector - is a nitrogen selective version of the thermionic detector, first described by Karmen and Giuffrida (1964). It consists of a rubidium bromide crystal in a normal flame, which leads to an enhanced response when nitrogen containing molecules and also phosphorous containing molecules enter the zone between the flame jet and the salt. The rubidium bromide evaporates and rubidium atoms react with the nitrogen containing molecules (Aue et al., 1967). The operating principle of the detector is probably based on the Lassaigne reaction, as all thermionic detectors give selectivity for those hetero-elements which can be identified by the Lassaigne test (Hewlett - Packard, 1970;

Riedmann, 1972b). The detector responds proportionally to the percent nitrogen content of the organic compound, and is claimed to be independent of the chemical structure (Hewlett - Packard, 1970). An important factor for proper detector operation is a high concentration of hydrogen in the flame (hydrogen flow rate 25 - 30 ml/min, air flow rate 180 - 220 ml/min). Optimization of the sensitivity and selectivity towards nitrogen containing compounds can be achieved further by selecting the proper collector salt position, which is variable in the Hewlett - Packard 15161A nitrogen detector. The combined characteristics of sensitivity, selectivity and wide linear dynamic range have been proved to be very useful in the analysis of some nitrogen containing compounds in biological fluids (Donike et al., 1970). Less time consuming sample clean-up procedures have been accomplished by using this detector (Riedmann, 1972a). A further advantage is that a minimum of solvent peak tailing, due to the detector selectivity, allows fast elution of the compounds on relatively short columns. Liquid phase bleeding even at elevated column temperatures is suppressed by the detector selectivity. High resolution columns are not generally required, because of the high signal suppression of sample endogenous material and strong enhancement of the nitrogen compound signal, resulting in a "separation by the detector" rather than by the column (Riedmann, 1973). According to the same author a 1 p.p.m. signal of a nitrogen containing compound is only interfered with by 2% or less in signal by 1000 p.p.m. of a non-nitrogen containing compound of the same retention time.

The experience gained with the use of the nitrogen detector in the present investigations on the determination of barbiturates in plasma, definitely endorses the above mentioned advantages. On all occasions a single partition step between the plasma sample and the extraction solvent allowed the quantitative measurement of less than 0.1 μg barbiturate per ml plasma. No purification of the plasma samples or derivative formation was necessary, except in the case of cyclobarbital where an endogenous plasma constituent produced interference. With hexobarbital a comparison has been made between normal flame ionization detection and the nitrogen detector, which illustrates the high increase in sensitivity and selectivity using the nitrogen detector (Breimer and van Rossum, 1974; chapter 2, Section II). The linear calibration curves which were obtained, were highly reproducible and the calculation factor always remained the same in the case of hexobarbital, methohexital, butobarbital and cyclobarbital. With vinylbital and heptabarbital there was some variation with time, so that it was necessary to construct a calibration curve for each sample series. The reason for this variation is not clear, but it is probably due to a changing sensitivity of the detector, which is slightly different for the barbiturate in question and the internal standard.

It should be mentioned here that the nitrogen detector is more complicated to handle than the normal flame ionization detector, as gas flow rates, the condition and the position of the rubidium salt are quite critical with respect to optimal performance. Gough and Sugden (1973) have studied the stability of this detector and they have concluded that variations in response of the nitrogen detector are higher than those of a normal flame detector. However, when working with internal standards and careful manipulation of this detector, it must be concluded that highly satisfactory results can be achieved.

EXTRACTION PROCEDURE

Barbiturates are weakly acidic drugs with pK_a-values ranging from 7.4 to 8.3 (Table I). The unionized form is lipophilic and it is in this form that they pass through lipoid membranes, such as the blood-brain and other blood-tissue barriers. It has been shown that the rate of transfer depends upon the lipid solubility of the undissociated molecule (Brodie et al., 1960; Mark, 1963). The true partition coefficient (TPC) is a measure for the lipophility of unionized drug, whereas the apparent partition coefficient (APC) is generally used to indicate the situation at physiological pH of 7.4 (Table I). The proportion of barbiturates in the aqueous phase at this pH is dependent upon the pK_a values. Nevertheless it can be seen that with all compounds there is still a high affinity for the organic phase under these circumstances. There is also a variation in lipophility between the barbiturates used in the present study. Organic solvents with different polarity can be applied therefore for the extraction of these compounds from plasma and urine, depending on their relative lipophility. Hexobarbital and methohexital for example, which are the compounds with the highest partition coefficients, could be extracted satisfactorily by the strongly apolar solvent light petroleum. A small amount of amylalcohol had to be added to increase polarity, so that reasonable recoveries were obtained (Brodie et al., 1953; Cooper and Brodie, 1955). For the less lipophilic barbiturates the more polar solvent diethylether or a mixture of diethylether, light petroleum and propanol have been used. Chloroform is often chosen for the extraction of drugs from biological fluids, but it gives rise to the formation of emulsions. Light petroleum offers the advantage that the extraction time on the whirlmixer is not very critical, while extractions with other solvents which last longer than 5 sec occasionally give interfering peaks with a concomitant increase of extracted endogenous substances. With cyclobarbital an interfering peak always appeared and therefore this compound had to be derivatized (methylated) prior to gas chromatography.

TABLE I. Physico-chemical properties of the barbiturates and the extraction solvents used in this study.

Compound	pK_a [1]	APC at pH 7.4 [2] (chloroform)	TPC [2]	Extraction solvent
Hexobarbital	8.3	111 [4]	129	Light petroleum 100/ amylalcohol 2
Methohexital	8.3 [3]	95 [3]	110	Light petroleum 100/ amylalcohol 2
Heptabarbital	7.45	18.2	36.6	Light petroleum 49/ diethylether 49/ n-propanol 2
Vinylbital	7.89	12.0	15.5	Diethylether
Butobarbital	7.86	11.9 [4]	15.5	Diethylether
Cyclobarbital	7.51	5.8	10.5	Diethylether

[1] According to Doornbos and de Zeeuw (1969).

[2] APC = apparent partition coefficient; TPC = true partition coefficient. The partition of the barbiturates between phosphate buffers of various pH-values (6.5 - 10.5) and chloroform was determined. The volume of both phases was 5.0 ml and the total amount barbiturate added was 50 μg. The concentration in both phases after shaking the mixture until equilibrium, was measured by gas chromatography. The percentage barbiturate in the organic phase was plotted against the pH and from the pH_{50} (50% in chloroform and 50% in the water phase) the TPC was calculated, while: $pH_{50} = pK_a + \log(TPC - 1)$; the APC at pH 7.4 $= \frac{TPC}{1 + 10^{7.4 - pK_a}}$

[3] According to Bush et al. (1966); the partition coefficients in this study were determined by using 1-chlorobutane as organic phase. The TPC of hexobarbital between this solvent and the aqueous phase was found to be 15. It must be concluded therefore that methohexital is the most lipophilic barbiturate found in the present investigation.

[4] According to Yih (1970).

In the present study no systematic approach was made for the extraction of the barbiturates from plasma or urine. In each case several solvent systems were tried and the one with the best result, with respect to reproducibility, was chosen. The same applies to the choice of the internal standard, of which hexo-

barbital often proved to be very suitable. For each compound the assay procedure will be described in detail in the following chapters.

GAS CHROMATOGRAPHIC CONDITIONS

Since the gas chromatographic conditions have been almost the same for all compounds studied, details will be given here and they will not be mentioned separately in each of the following chapters of this Section. Some information has been summarized in Table II.

TABLE II. Gas chromatographic conditions of the barbiturates investigated.

Compound	Stationary phase	Column temperature	Retention time (min)	Internal standard
Hexobarbital	OV-17 3%	230° (240°)	3.6 (2.3)	Methohexital
3-Keto-hexobarbital	OV-17 3%	240°	6.4	Codeine (E.S.)[2]
Methohexital	OV-17 3%	230°	2.3	Hexobarbital
Heptabarbital	OV-17 3%	240°	4.2	Hexobarbital
Vinylbital	OV-17 3%	240°	1.5	Hexobarbital
Butobarbital	OV-17 3%	240°	1.3	Hexobarbital
Cyclobarbital	OV-17 3%	220°	2.6[1]	Cyclopal

[1]) After flash-heater methylation by trimethylaniliniumhydroxide.
[2]) E.S. = external standard.

Apparatus. A Hewlett - Packard, Model 5750 gas chromatograph, equipped with a nitrogen detector (rubidium bromide; Hewlett - Packard, Model 15161A), was used.

Column. A glass column (1.8 m x 3 mm I.D.), packed with 3% OV-17 on Gas-Chrom Q, 60 - 80 mesh (Applied Science Lab., State College, Pa., U.S.A.) was used. The retention times of the compounds are given in Table II.

Temperatures. The temperatures of the column for each barbiturate are given in Table II. The temperature of the injection port was maintained at 280° and the detector at 380°.

Gas flow-rates. The carrier gas was helium at a flow-rate of 25 - 30 ml/min. In order to obtain optimal performance of the nitrogen detector, an auxiliary stream of carrier gas at a flow-rate of 20 - 30 ml/min was led directly into the

detection system. The hydrogen flow-rate was 30 ± 0.5 ml/min and the air flow-rate 200 ± 10 ml/min. A very constant hydrogen flow-rate was required for the nitrogen detector and this was achieved by using a differential flow controller before the normal flow control of the gas chromatograph.

Operation of the nitrogen detector. The distance between the collector, containing the rubidium bromide crystal, and the flame is very important with respect to the achievement of high sensitivity and selectivity with nitrogen-containing compounds. At the beginning of each day, the collector was moved step by step in the direction which gave the maximum ionization current (maximum recorder deflection, range 10^3, attenuation 32). The crystal was cleaned twice a week by carefully wiping it with a soft brush.

CONCLUDING REMARKS

Finally it must be emphasized that the methods described here have been developed as a research tool to measure low levels of barbiturates, without interference from endogenous materials. No guarantee is offered that other drugs will not interfere, or that mixtures of several barbiturates will well separate under these gas chromatographic conditions. For information concerning the separation and quantitative determination of several barbiturates in toxicological cases, the reader is referred to the extensive study by Berry (1973).

It should further be mentioned that the identity of the compounds eluting from the gas chromatograph was checked with the combined gas chromatograph - mass spectrometer (LKB 9000).

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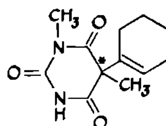
HEXOBARBITAL IN PLASMA AND URINE

INTRODUCTION

The structure of hexobarbital, an N-methylated barbiturate (1,5-dimethyl-5-(1-cyclohexenyl)-barbituric acid), is shown below. Due to the presence of the N-methylgroup at position 1, hexobarbital has a relatively high pK_a-value (8.19) and also it is highly lipophilic (Chapter 1, Section II). A further consequence of this N-methylation is that the compound contains an asymmetric centre at the 5-position (indicated with an asterisk in the structure). Knabe and Kräuter (1965) have separated the optical antipodes, but in practice the racemic mixture is used.



hexobarbital

In a recent article Bush and Weller (1972) have reviewed the physicochemical properties of hexobarbital and also the methodologies for its quantitative determination in biological fluids and tissues. Prior to 1965 U.V.-spectrophotometry was used, when the fate of the drug in animals or man was studied. More recently the compound has been discussed in studies concerning the gas chromatographic determination of barbiturates in general. None of these techniques, however, are very suitable for the determination of low hexobarbital levels, as has been outlined in Chapter 1 of this Section. Therefore a specific and sensitive gas chromatographic assay for hexobarbital was developed, using the nitrogen detector (Breimer and van Rossum, 1974). This detector allows the determination of very low levels, sufficient to study the pharmacokinetics of the drug after administration of therapeutic doses to man. Furthermore, a column chromatographic purification procedure has been developed, which can be applied when plasma samples from hepatitis patients contain high amounts of interfering endogenous materials.

MATERIALS AND METHODS

Reagents

Hexobarbital was obtained from Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands. Methohexital was obtained from Eli Lilly, U.S.A. (a gift of methohexital for reference purposes is greatfully acknowledged). Light petroleum, boiling range 40 - 60°, and amyl alcohol, were of analytical-reagent grade (E. Merck, Darmstadt, G.F.R.). A standard hexobarbital solution was prepared at a concentration of 1 mg per 100 ml of absolute ethanol (analytical-reagent grade, E. Merck). A standard methohexital solution was prepared at a concentration of 3 mg per 100 ml of absolute ethanol. Silica gel (Kieselgel 60 für Säulenchromatographie, 70 - 230 mesh) was obtained from E. Merck.

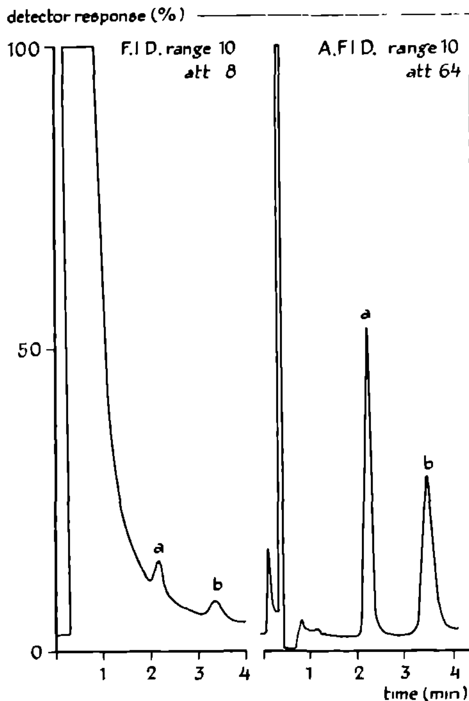


FIG. 1. Difference in response of a normal flame ionization detector (FID) and nitrogen detector (AFID) to methohexital (a) and hexobarbital (b) after injection of 20 ng of each.

Extraction procedure

Normal plasma. To 2.0 ml plasma in a conical tube was added 2 ml of distilled water and 0.1 ml of internal standard solution, containing 3 μg of methohexital. The mixture was extracted twice with 10 ml of light petroleum-amyl alcohol (100 : 2) on a Cenco whirlmixer. Mixing was carried out vigorously for 5 - 10 sec (twice). The upper organic layer was removed each time with a Pasteur pipette and transferred into a conical evaporation tube. The solvent was evaporated to dryness at 40° in a stream of dry air. The residue was dissolved in 0.1 ml of absolute ethanol and 2 - 5 μl of this solution were injected into the gas chromatograph using a Hamilton syringe.

Hepatitis plasma. Plasma or serum samples from hepatitis patients sometimes showed strong interference with the hexobarbital and methohexital peaks in the gas chromatogram (Fig. 3). Simple purification was obtained by transferring the light petroleum-amyl alcohol extract in 0.2 - 0.4 ml of absolute ethanol into a silica gel column (I.D. 8 mm, height 4 cm) and eluting with absolute ethanol.

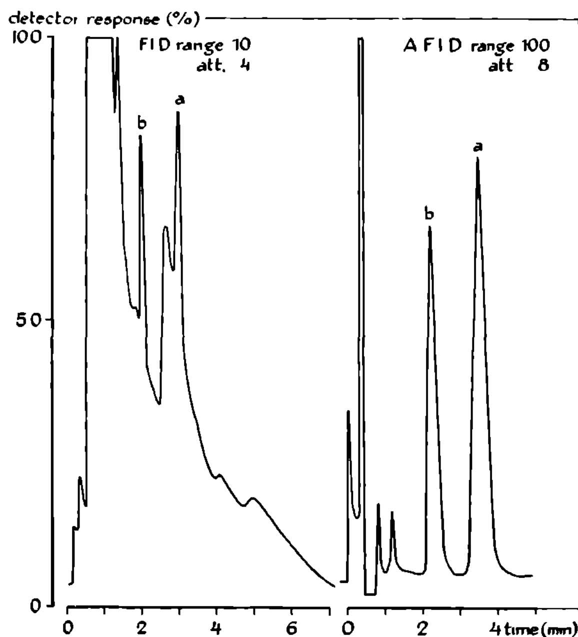


FIG. 2. Gas chromatograms of a 2-ml plasma extract (FID and AFID) obtained from a subject 4 h after receiving 600 mg of hexobarbital orally. Injection volume 2 μl. a, hexobarbital (concentration in plasma 2.1 μg/ml); b, methohexital (internal standard; concentration in plasma 1.5 μg/ml).

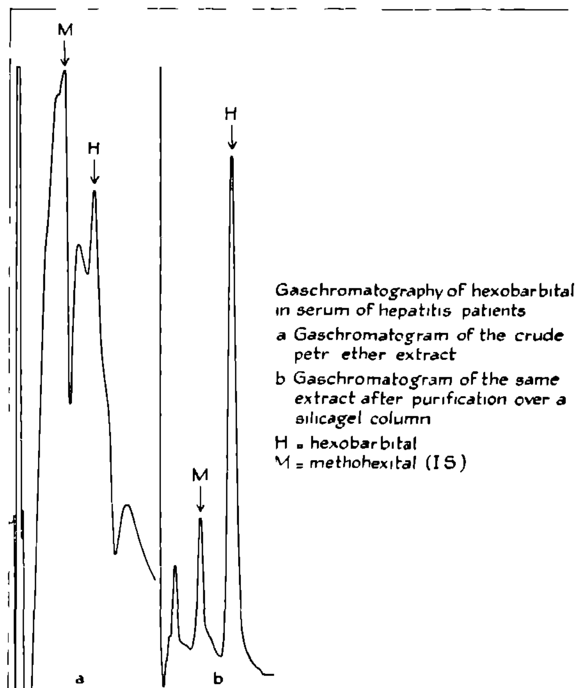


FIG. 3. Gas chromatography of hexobarbital in serum of hepatitis patients with a high bilirubin content: a, gas chromatogram of the crude light petroleum extract; b, gas chromatogram of the same extract after purification through a silica gel column. H = hexobarbital; M = methohexital (internal standard, 1.5 μg/ml plasma).

The first 3 ml of eluate were collected and then concentrated to a volume of 0.1 ml; this solution was analyzed by gas chromatography. With the aid of calibration graphs, it was verified that the peak area ratio of hexobarbital to internal standard was not changed by the purification procedure.

Urine. Although very little hexobarbital is excreted into the urine unchanged, its renal excretion rate and cumulative renal excretion were quantified in the following way. In a conical tube 10.0 ml urine were extracted twice on the whirlmixer with 20 ml light petroleum-amyl alcohol (100 : 2). The combined organic layers were evaporated to dryness and the residue was dissolved in 0.1 ml of absolute ethanol, containing 8 μg cyclobarbital (external standard). This solution was used for injection into the gas chromatograph.

Gas chromatography

As described in Chapter 1, Section II.

Preparation of calibration graphs

The concentration of hexobarbital in plasma and urine samples was calculated with the aid of calibration graphs, which were prepared by adding known amounts of hexobarbital to 2.0 ml of blank plasma or 10.0 ml of blank urine. The samples were analyzed by the same procedure described above and the ratio of the peak areas of hexobarbital to internal or external standard was plotted against known concentrations of hexobarbital.

Recovery studies

Recoveries of hexobarbital at different concentrations were determined by adding known amounts of hexobarbital to 2.0 ml of blank plasma. After extraction, 3.0 μg of methohexital were added and the relative peak area ratio (R_I) was calculated. This value was compared with the ratio (R_{II}) obtained by GC of the same standard amount of hexobarbital with 3.0 μg of methohexital:

$$\text{Recovery (\%)} = \frac{R_I}{R_{II}} \times 100.$$

RESULTS AND DISCUSSION

Gas chromatographic sensitivity and selectivity

In Fig. 1, typical gas chromatograms are shown of hexobarbital and methohexital standards, obtained both by normal flame ionization detection (FID) and by nitrogen-selective detection (AFID). A large increase in sensitivity is evident when using the nitrogen detector. Barbiturates contain two nitrogen atoms, and as the detector signal is proportional to the nitrogen equivalent in the molecule, the presence of two nitrogen atoms favours high sensitivity. The position of the rubidium bromide crystal with respect to the flame and the gas flow rates are important factors in obtaining a high N-C ratio and the influence of these factors has been extensively studied (Donike et al., 1970; Karmen, 1969). The negative detector response shortly after injection has been observed

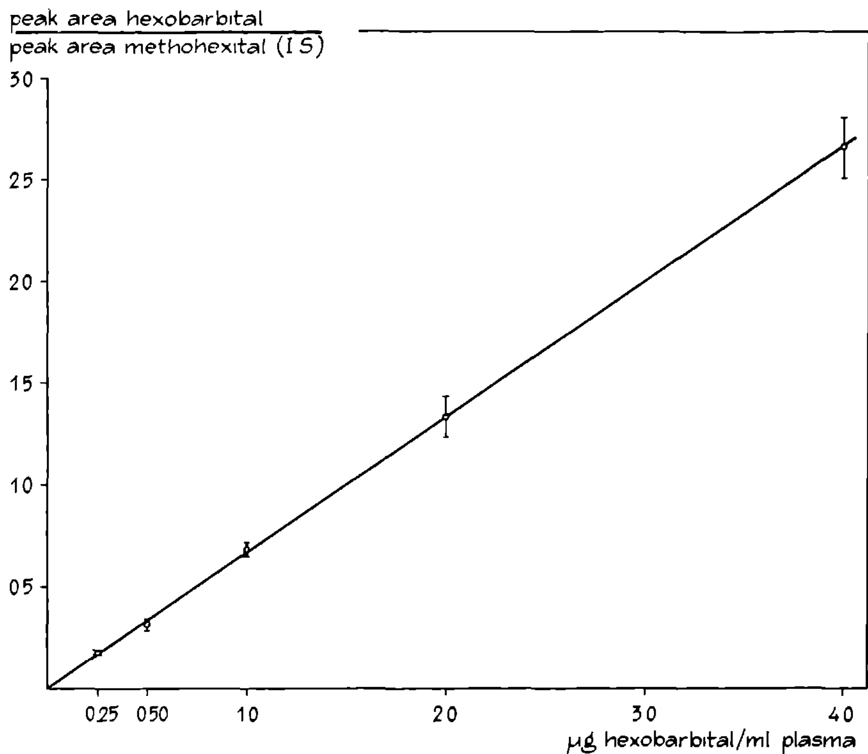


FIG. 4. Calibration graph for the determination of hexobarbital in plasma, using methohexital as internal standard (1.5 μg/ml). Mean values and standard deviations of at least six determinations.

by several investigators using a nitrogen detector (Gerhardt and Aue, 1970). The detection limit is about 0.5 ng per single injection for the two barbiturates under investigation.

In Fig. 2, typical gas chromatograms are shown of a plasma extract containing hexobarbital and methohexital. With the normal FID, there is interference of a plasma peak with the hexobarbital. However, when using the nitrogen detector there is a large increase in selectivity towards the nitrogen-containing compounds. There is hardly any solvent peak and the base line has become horizontal. Time-consuming purification would have been necessary if normal FID had been used, whereas the extract can be analyzed directly by using the nitrogen detector. Purification is required only in some cases of

hepatitis patients with very high plasma bilirubin contents. This can be achieved satisfactorily by running the extracts through a silica gel column (Fig. 3). Concentrations in plasma down to 50 ng/ml can be determined.

Extraction procedure and precision

The extraction by light petroleum-amyl alcohol (100 : 2) was chosen as it is suitable for the highly lipophilic barbiturates hexobarbital and methohexital (both N-methylated). Cooper and Brodie (1955) already have used this solvent system for the extraction of hexobarbital from biological material. A small amount of amyl alcohol had to be added in order to get a reasonable recovery.

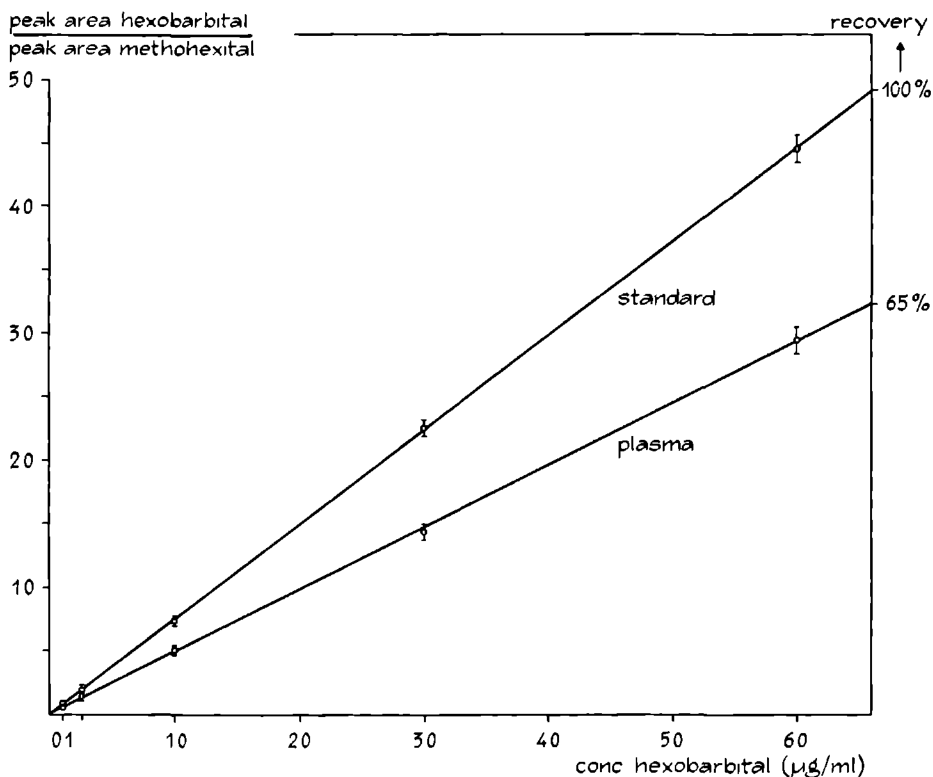


FIG. 5. Peak area ratio of hexobarbital to methohexital as a function of known hexobarbital concentrations for the determination of recovery. Mean values and standard deviations ($n = 4$).

The only disadvantage of amyl alcohol was an impurity that eluted from the gas chromatograph a few minutes after hexobarbital. Two samples in succession could be analyzed and then it was necessary to wait for the two "amyl alcohol" peaks. Later on this peak was no longer present, when using a different lot of amyl alcohol. No interfering plasma constituents are extracted, except in some cases with hepatitis plasma, and the formation of an emulsion never occurred. Vigorous extraction for 5 - 10 sec (twice) gives highly reproducible results. From the calibration curve in Fig. 4 which is composed of at least six individual calibration curves prepared on different occasions over a period of 1 year, it can be deduced that the procedure is highly reproducible and has a good precision over a large concentration range. Standard deviations did not exceed $\pm$ 5%, except at the 2.0 μg level ($\pm$ 7%).

The extraction yields (recoveries) were determined in the same concentration range as those encountered in practice and they appeared to be constant, with a mean value of 65% (Fig. 5). Standard deviations were in the range 3 - 5% ($n = 4$). Although in general an extraction should give a high yield, in this particular instance the low detection limit and the good reproducibility permit the use of light petroleum-amyl alcohol as the extraction solvent.

The size of the plasma samples was 2.0 ml throughout the investigation, but it is reasonable to assume that smaller samples can also be analyzed satisfactorily by this method. This could be of advantage in clinical situations where the sample size is a limiting factor.

CONCLUSION

This method for the assay of hexobarbital in plasma is rapid, sensitive and of good precision. It is therefore suitable for pharmacokinetic studies in man and also in patients with liver disease.

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3-KETO-HEXOBARBITAL IN URINE

INTRODUCTION

3-Keto-hexobarbital is an important metabolite of hexobarbital, which has been identified in the urine of several species. The structure of this compound is shown in Fig. 1. Bush et al. (1953) were the first to identify this compound in the urine of dogs after hexobarbital administration. Tsukamoto et al. (1956a; 1957) have reported the isolation of 3-keto-hexobarbital from rabbit urine and human urine. This compound is formed also during hexobarbital metabolism in mice (Noordhoek and Gerritsen, 1969; Gerber et al., 1971) and in rats (Holcomb et al., 1969; Yih, 1974). Other important metabolites of hexobarbital, are 3-hydroxy-hexobarbital, 3-keto-norhexobarbital and barbiturate-ring cleavage products (Bush and Weller, 1972). None of the oxidized metabolites has hypnotic properties (Bush et al., 1953; Yoshimura, 1958). There appear to be no quantitative studies available on the excretion of metabolites after hexobarbital administration in man.

When studying the pharmacokinetics of hexobarbital in man, it was considered of interest to measure the renal excretion of one or more metabolites simultaneously. With respect to the comparison of the fate of hexobarbital antipodes in the same subject, this would yield additional information about possible different metabolic pathways. Also in patients with impaired liver function it is interesting to determine whether the metabolite pattern is changed in comparison with healthy controls. It was attempted to develop a gas chromatographic method for the quantitative determination of 3-keto-hexobarbital and of 3-hydroxy-hexobarbital in human urine. Unfortunately the hydroxy-compound could not be determined successfully. Norhexobarbital was omitted because it is only excreted in trace amounts. A simple and sensitive assay for the determination of 3-keto-hexobarbital in urine is described below, which involves a single partition step between urine and ethyl acetate, followed by gas chromatography with nitrogen selective detection. Gas chromatographic procedures for the quantitative determination of hexobarbital metabolites in

biological fluids have not been reported previously. Tsukamoto et al. (1958) have measured hexobarbital metabolites in urine by the use of buffered paper chromatography in conjunction with UV-spectrophotometry. Anders and Latorre (1970) have included hexobarbital and 3-keto-hexobarbital in a study on the applicability of high-pressure liquid chromatography to the resolution of mixtures of barbiturates and hydantoins. The employment of this procedure to biological fluids has not been published yet.

MATERIALS AND METHODS

Preparation of 3-keto-hexobarbital

The compound was prepared according to the method of Tsukamoto et al. (1956). To a stirred suspension of 5 g hexobarbital in 40 ml acetic anhydride was added dropwise a solution of 5.6 g chromium trioxide in 40 ml acetic anhydride, during 1 h. The reaction mixture was stirred at 35° for 1 h and then allowed to stand overnight. The solvent was evaporated completely under reduced pressure and 100 ml water was added to the residue. This was extracted three times with 100 ml ethyl acetate and the combined extracts were dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the residue was repeatedly recrystallized from methanol, until gas chromatographically pure 3-keto-hexobarbital was obtained. Calculated for $C_{12}H_{14}O_4N_2$: C, 57.59; H, 5.64; N, 11.20; Found: C, 57.61; H, 5.77; N, 10.93.

Extraction procedure

In a conical tube 2.0 ml urine was extracted twice with 10 ml of distilled ethyl acetate (analytical-reagent grade, E. Merck) on a Cenco whirlmixer during 10 sec. The combined organic layers were evaporated to dryness and 60 μg codeine, dissolved in 0.1 ml absolute ethanol, was added to the residue as an external standard. After evaporation of the alcohol the residue was dissolved in about 0.5 ml acetone (analytical-reagent grade, E. Merck) and 5 μl of this solution were injected into the gas chromatograph.

Gas chromatography

As described in Chapter 1, Section II.

Preparation of calibration graphs

The concentration of 3-keto-hexobarbital in urine samples was calculated with the aid of calibration graphs, which were prepared by adding known amounts of 3-keto-hexobarbital to a 2.0-ml blank urine sample. The samples were analyzed by the same procedure described above and the ratio of peak areas of 3-keto-hexobarbital to external standard was plotted against known concentrations of 3-keto-hexobarbital.

RESULTS AND DISCUSSION

Since 3-keto-hexobarbital is not commercially available, the compound had to be synthesized for reference purposes. During the oxidation of hexobarbital with chromium trioxide not only the 3-keto-compound is formed, but also some 6-keto-hexobarbital. Due to possible sterical hindrance of the 6-position, the yield of this compound is only about 10% of the amount of 3-keto-hexobarbital that is formed (Tsukamoto et al., 1956b). The two compounds are well separated on the OV-17 column, the retention time of 6-keto-hexobarbital is a little less than the 3-keto-isomer. The pure 3-keto-hexobarbital was obtained after recrystallization. In Fig. 1 the mass spectra of the two isomers (LKB 9000 GC-MS combination, ionization potential of 20 eV), are given. Distinct differences in mass spectrometric fragmentation behaviour can be observed. With 6-keto-hexobarbital the molecular ion (m/e 250) is also the base peak, whereas with the 3-keto-compound the cyclohexenone fragment (m/e 95) and the M-15 fragment (loss of the methyl group at C5) are the major peaks, which have about the same intensity. The metastable ion at about m/e 221 is also characteristic of the 3-keto-compound. Further fragmentation behaviour can be explained in accordance with previously published data on the mass spectrometric behaviour of barbiturates (Gilbert et al., 1970; Skinner et al., 1973).

Analysis of a urine sample, by means of GC - MS, from a human subject who had taken hexobarbital, showed that one of the compounds eluting from the gas chromatograph was identical to synthetic 3-keto-hexobarbital (Fig. 1). No 6-keto-hexobarbital could be identified in human urine, whereas norhexobarbital was found to be excreted only in trace amounts. Also small amounts of unchanged drug were detected. In Fig. 2 the gas chromatogram of a urine extract, containing 3-keto-hexobarbital, is shown. Most peaks which appear before this hexobarbital metabolite, also show up after extraction of blank urine. The peak eluting with a retention time of 5.4 min probably represents another hexobarbital metabolite, but its structure has not been elucidated yet. Ethyl

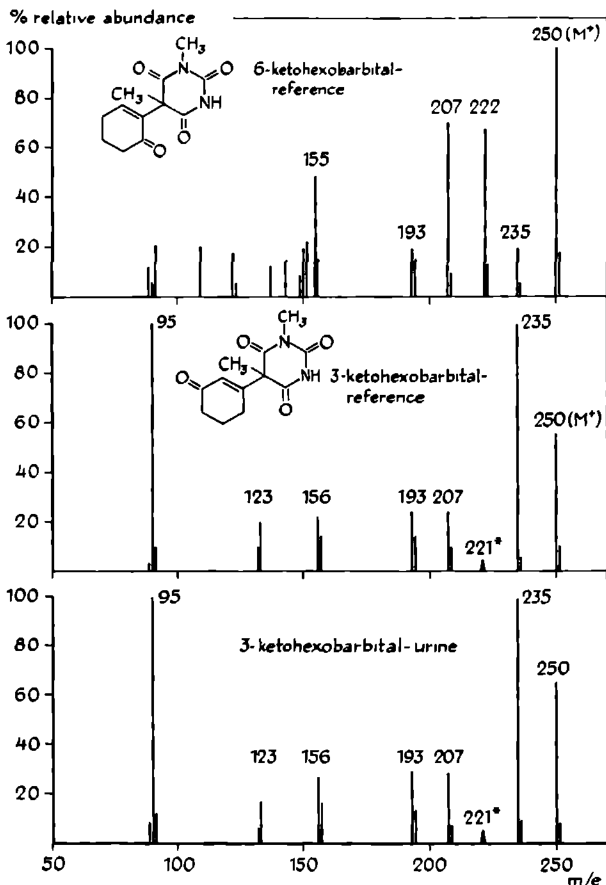

FIG. 1. Mass spectra of synthetic 6-keto-hexobarbital, synthetic 3-keto-hexobarbital and biological 3-keto-hexobarbital (LKB 9000 gas chromatograph - mass spectrometer combination). The biological material was extracted from urine of a subject who had received 600 mg of hexobarbital orally.

acetate has often been used for the extraction of the rather polar barbiturate metabolites from urine (Bush and Weller, 1972). The recovery of 3-keto-hexobarbital from urine by the double extraction with ethyl acetate was approximately 86% (determined at the same concentrations as in the calibration graph of Fig. 3).

The choice of an internal or external standard has proved difficult in the assay of this metabolite. Several compounds, e.g. barbiturates and related

substances, have been tried. However, most of these had shorter retention times than 3-keto-hexobarbital. This was undesirable since interference with endogenous materials was liable to occur (Fig. 2). Other compounds showed extensive peak tailing on a 3% OV-17 column and were therefore excluded. Finally it was decided to use codeine (free base), because this compound appeared to have an appropriate retention time and linear calibration graphs were obtained over a great concentration range. Naturally this substance, with basic properties, cannot be used as an *internal standard for the determination* of weakly acidic drugs such as barbiturates. Therefore it is added after extraction and must be considered as an external standard. Although the choice of codeine cannot be considered as ideal, satisfactory results have been obtained. In Fig. 3 calibration curves for the determination of 3-keto-hexobarbital in urine are illustrated, using codeine as standard. The results revealed that the ratio between 3-keto-hexobarbital and the external standard at a certain concentration, varied slightly in the time. This might be due to selective changes

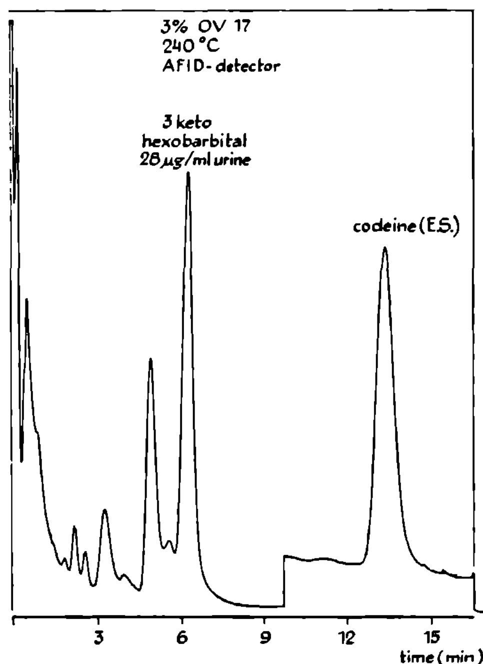

FIG. 2. Gas chromatogram of a 2-ml urine extract obtained from a subject 6 h after receiving 600 mg of hexobarbital orally. Codeine base was used as external standard (concentration 30 μg/ml urine).

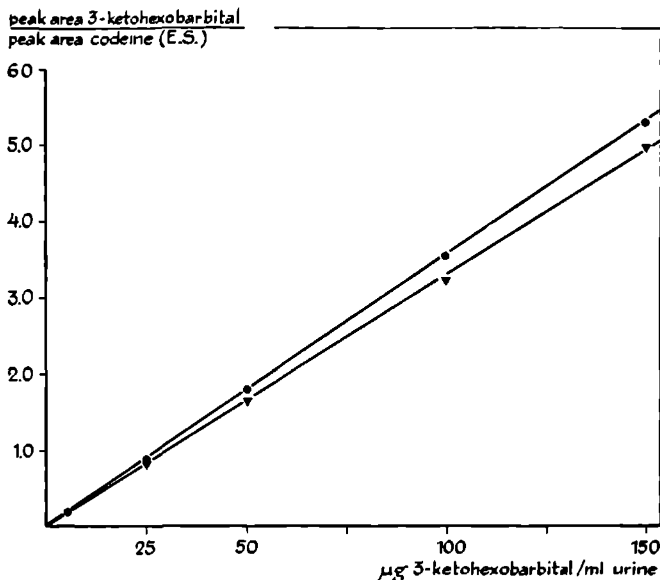

FIG. 3. Calibration graphs for the determination of 3-keto-hexobarbital in urine, prepared on different occasions, using codeine base as external standard (30 µg/ml urine).

in the sensitivity of the nitrogen detector towards one of the two compounds. The same phenomenon has been observed in the assay of vinylbital and heptabarbital in plasma. The variation was in the order of 10 - 15%, so that the preparation of a calibration graph was necessary for each day when a series of urine samples had to be analyzed for 3-keto-hexobarbital. This disadvantage is only of minor importance, as it must be realized that the preparation of a calibration graph does not take much extra time. Concentrations down to 5 µg/ml urine can be determined by this method.

It should be mentioned that strongly varying results were obtained initially, when the extraction residue was "dissolved" in absolute ethanol. It appeared that 3-keto-hexobarbital was poorly soluble in ethanol and therefore acetone was used. A relatively large amount of acetone is required, otherwise dissolution problems arise.

CONCLUSION

This method for the quantitative determination of 3-keto-hexobarbital in urine is reliable, rapid and sensitive. It is specific and less time consuming than

the methods described previously for the quantitative determination of hexobarbital metabolites. A calibration curve should be prepared with each sample series.

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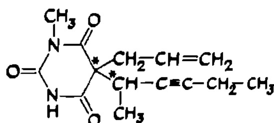
METHOHEXITAL IN PLASMA

INTRODUCTION

Methohexital (α-dl-1-methyl-5-allyl-5-(1-methyl-2-pentynyl)-barbituric acid) is a N-methylated compound, the structure of which is shown below. The allyl-group and pentynyl-group also considerably contribute to its great lipid solubility. Bush et al. (1966) have compared the partition coefficients of several barbiturates, including hexobarbital, thiopental and methohexital between buffers of different pH and 1-chlorobutane. They have reported the highest lipophility for methohexital. The compound contains two asymmetric centres (indicated by asterisks in the structure) and four stereoisomers are possible. In practice the α-dl-racemic mixture is used, because the β-d and β-l optical isomers produce excessive motor activity (Gibson et al., 1959).



methohexital

Previous studies on the physiologic disposition of methohexital in man after administration of high doses have been performed with the aid of UV-spectrophotometry (Brand et al., 1963) or gas chromatography with flame ionization detection (Sunshine et al., 1966). Both methods, however, lack the sensitivity to measure plasma levels for a certain period following administration of therapeutic doses of the compound. Therefore these cannot be used in pharmacokinetic studies and a more sensitive assay had to be developed. A suitable method is described in this chapter, employing gas chromatography with nitrogen selective detection.

MATERIALS AND METHODS

Reagents

Methohexital was obtained from Eli Lilly, U.S.A. (a gift of this compound for reference purposes is greatfully acknowledged). Hexobarbital was obtained from OPG, Utrecht, The Netherlands. Light petroleum, boiling range 40 - 60°, and amyl alcohol, were of analytical-reagent grade (E. Merck). A standard methohexital solution was prepared at a concentration of 1 mg per 100 ml of absolute ethanol (analytical-reagent grade, E. Merck). A standard hexobarbital solution was prepared at a concentration of 3 mg per 100 ml of absolute ethanol.

Extraction procedure

To 2.0 ml plasma in a conical tube was added 2 ml of distilled water and 0.1 ml of internal standard solution, containing 3 μg of hexobarbital. The mixture was extracted twice with 10 ml of light petroleum - amyl alcohol (100 : 2) on a Cenco whirlmixer. Mixing was carried out vigorously for 5 - 10 sec (twice). The upper organic layer was removed each time with a Pasteur pipette and transferred into a conical evaporation tube. The solvent was evaporated to dryness at 40° in a stream of dry air. The residue was dissolved in 0.1 ml of absolute ethanol and 2 - 5 μl of this solution were injected into the gas chromatograph.

Gas chromatography

As described in Chapter 1, Section II.

Preparation of calibration graphs

The concentrations of methohexital in plasma were calculated with the aid of calibration graphs, which were prepared by adding known amounts of methohexital to 2.0 ml of blank plasma. The samples were analyzed by the same procedure described above and the ratio of the peak areas of methohexital to internal standard was plotted against known concentrations of methohexital.

RESULTS AND DISCUSSION

The assay for methohexital in plasma is in fact the same as for hexobarbital (Chapter 2 of this Section). When studying the determination of hexobarbital

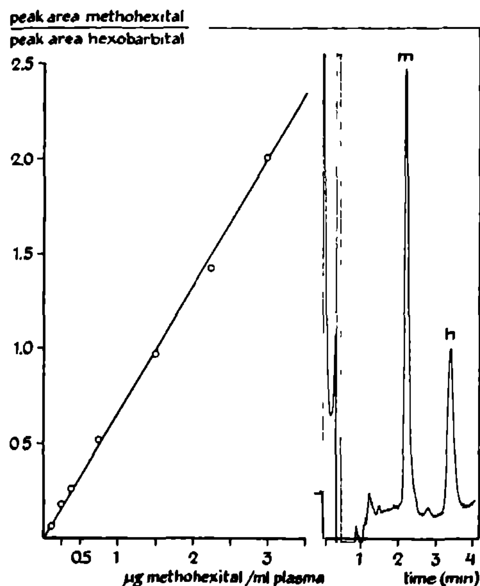


FIG. 1. *Left.* Calibration graph for the determination of methohexital in plasma, using hexobarbital as internal standard (1.5 μg/ml plasma). *Right.* Gas chromatogram of a 2-ml plasma extract obtained from a patient 10 min after receiving 200 mg of methohexital sodium intravenously. m = methohexital (concentration 3.8 μg/ml plasma), h = hexobarbital (internal standard, concentration 1.5 μg/ml plasma).

in plasma, methohexital proved to be a very reliable internal standard. In the present assay hexobarbital is used as internal standard. The entire procedure appears to give equally good results for the two compounds. In Fig. 1 a gas chromatogram is shown of a plasma extract containing methohexital. There is no interference with other peaks and the nitrogen detector permits the determination of very low levels (detection limit approximately 50 ng/ml plasma). Sensitivity is at least 50-fold greater as compared with normal flame ionization detection (Sunshine et al., 1966). The short retention times of the compounds on the 3% OV-17 column and the rather simple extraction procedure make it possible to analyze many samples in a relatively short period of time. The slope of the calibration graphs (example given in Fig. 1) was always the same and equal to the slope of the hexobarbital calibration graphs. An extraction recovery of about 65% was found for methohexital.

CONCLUSION

This method for the assay of methohexital in plasma is rapid, sensitive and reproducible. It is therefore suitable for pharmacokinetic studies in man, when therapeutic doses are administered.

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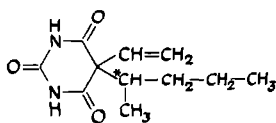
VINYLBITAL IN PLASMA AND URINE

INTRODUCTION

Vinylbital (5-vinyl-5-(1-methylbutyl)-barbituric acid) is a barbiturate containing an asymmetric centre in the side chain, the structure being illustrated below. Knabe and Kräuter (1965) have described a procedure for the separation of the optical isomers, but in practice the racemic mixture is used. The compound has moderate lipophilic properties in comparison to hexobarbital and methohexital.



vinylbital

No specific and sensitive method for the quantitative determination of vinylbital in biological fluids has been reported previously in the literature. Therefore such a method was developed, using gas chromatography with nitrogen selective detection, for the study of the pharmacokinetics of vinylbital in man.

MATERIALS AND METHODS

Reagents

Vinylbital was obtained from BYK-Nederland, Zwanenburg, The Netherlands (a gift of vinylbital for reference purposes is greatfully acknowledged). Hexobarbital was obtained from OPG, Utrecht, The Netherlands. Diethyl ether, light petroleum boiling range 40 - 60° and propanol-2 were of analytical-reagent grade (E. Merck). Diethyl ether was freshly distilled before use. Phosphate

buffer pH 6.0 was prepared by adding 61.5 ml of a solution containing 6.745 g $Na_2HPO_4 . 12 H_2O$/250 ml distilled water to 438.5 ml of a solution containing 4.54 KH_2PO_4/500 ml distilled water. A standard vinylbital solution was prepared at a concentration of 1 mg/100 ml absolute ethanol (analytical-reagent grade, E. Merck). A standard hexobarbital solution was prepared at a concentration of 2 mg/100 ml absolute ethanol.

Extraction procedure

Plasma. To 2.0 ml plasma in a conical tube was added 1.0 ml of phosphate buffer pH 6.0 and 0.1 ml of internal standard solution containing 2 μg hexobarbital. The mixture was extracted twice with 5 ml of diethyl ether on a Cenco whirlmixer. Mixing was carried out at half the maximum speed of the whirlmixer (about 20 r.p.s.), twice, during 3 - 4 sec (this period was quite critical, since longer extraction resulted in the concomitant extraction of interfering endogenous plasma substances). The combined organic layers were evaporated to dryness in a stream of dry air. The residue was dissolved in 0.1 ml of absolute ethanol and 2 - 5 μl of this solution were injected into the gas chromatograph.

Urine. To 10.0 ml urine in a conical tube was added 0.1 ml of internal standard solution containing 2 μg hexobarbital. The mixture was extracted twice with 10 ml diethyl ether - light petroleum - propanol-2 (49 : 49 : 2) on a Cenco whirlmixer. Mixing was carried out at half the maximum speed of the whirlmixer, twice, during 10 sec. Further procedures were the same as described for plasma.

Gas chromatography

As described in Chapter 1, Section II.

Preparation of calibration graphs

The concentration of vinylbital in plasma and in urine samples was calculated with the aid of calibration graphs, which were prepared by adding known amounts of vinylbital to 2.0 ml of blank plasma or 10.0 ml of blank urine. The samples were analyzed by the same procedure described above and the ratio of the peak areas of vinylbital to internal standard was plotted against known concentrations of vinylbital.

RESULTS AND DISCUSSION

Several organic solvents were studied for the extraction of vinylbital from plasma. Many of these, however, gave interfering peaks of endogenous plasma materials in the gas chromatogram. Only light petroleum, alone or in combination with 2% amyl alcohol, gave completely clean gas chromatograms, but the extraction yield for the drug was too low when using these solvents. Initially extraction was carried out vigorously (maximum speed) on the whirlmixer for about 10 sec but unsatisfactory results were obtained. It was determined that the extraction time was critical with respect to the interference of endogenous substances. Mixing times greater than 5 sec at half the maximum speed gave interference, whereas the compounds to be analyzed had already been extracted

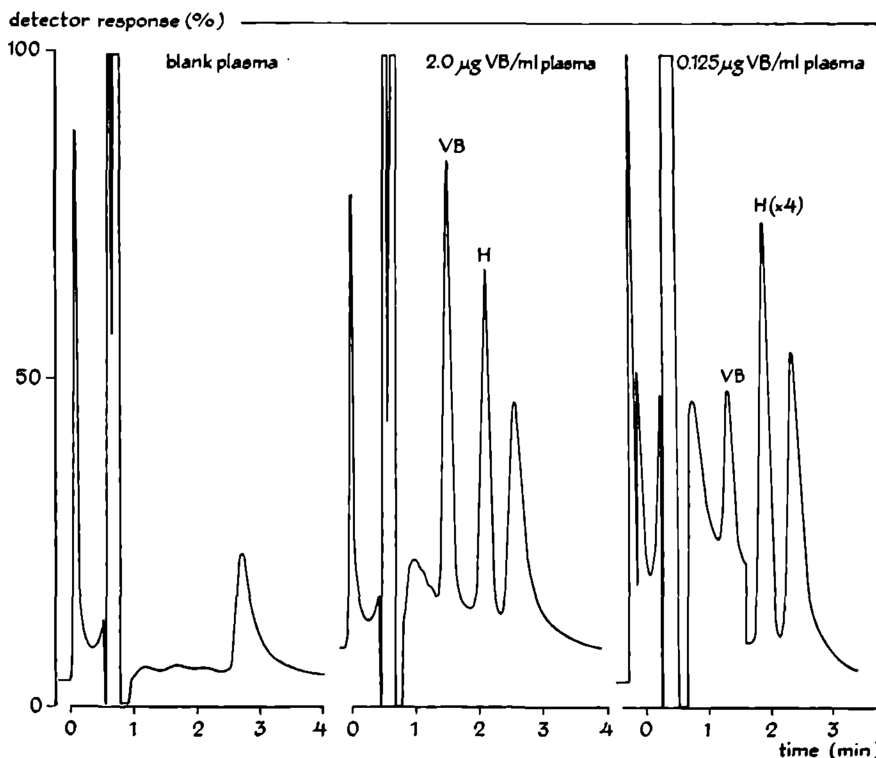


FIG. 1. Gas chromatograms of a 2-ml plasma extract obtained from a subject prior to *(left)* and 8 h after *(middle)* receiving 150 mg vinylbital orally. The *right* gas chromatogram was obtained after extraction of a 2-ml blank plasma sample to which 0.25 μg vinylbital (VB) had been added. Hexobarbital (H) was used as internal standard (concentration 1.0 μg/ml plasma).

sufficiently in this time. Another improvement was afforded by using 5 ml solvent instead of 10 ml and adding 1 ml of phosphate buffer to the sample before extraction. Finally, 3 - 4 sec were chosen as the optimal extraction time and gas chromatograms obtained in this manner are shown in Fig. 1. There still appears to be a plasma peak, but this does not interfere with vinylbital and hexobarbital and consequently the latter compound was chosen as internal standard. Vinylbital concentrations down to 50 ng/ml plasma can be measured.

A recovery of about 86% was found for vinylbital at each concentration, when the extraction lasted for 3 - 4 sec (Fig. 2). For hexobarbital this value was

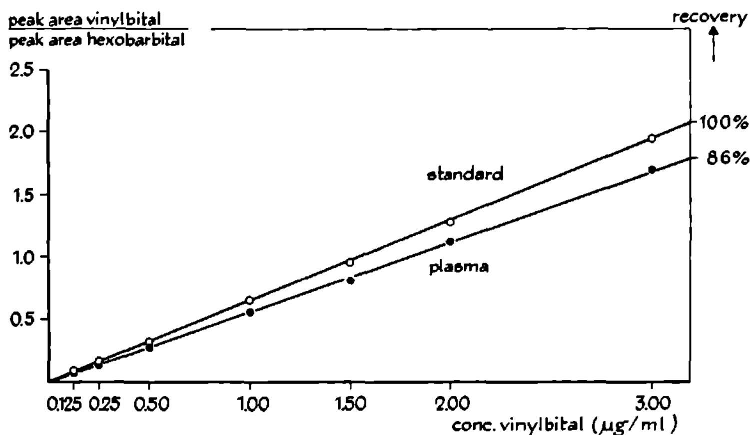


FIG. 2. Peak area ratio of vinylbital to hexobarbital as a function of known vinylbital concentrations for the determination of recovery. The plasma curve was obtained in the same way as described for the preparation of a calibration graph, except that 2.0 μg hexobarbital was always added after extraction. The standard curve was obtained by comparing known standard amounts of vinylbital to 2.0 μg hexobarbital.

91%. In Fig. 3 calibration curves for the determination of vinylbital in plasma are given and it appears that there is a little variation with time. This might be due to selective changes in sensitivity of the nitrogen detector towards one of the two compounds. Another possible explanation is that the extraction yield of vinylbital might vary slightly, as this is somewhat dependent on extraction time. It was necessary to prepare a calibration graph for each day when a series of samples had to be analyzed for vinylbital. This disadvantage is only of minor importance since it is not time consuming.

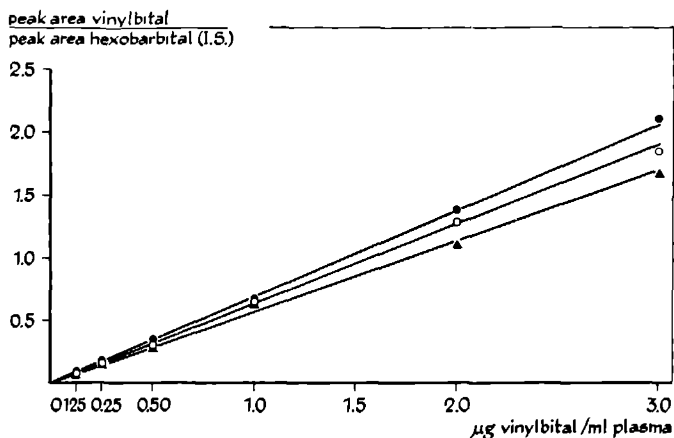


FIG. 3. Calibration graphs for the determination of vinylbital in plasma, prepared on different occasions, using hexobarbital as internal standard (1.0 μg/ml plasma).

With respect to the determination of unchanged vinylbital in urine, the best results were obtained with a mixture of diethyl ether and light petroleum, to which a small amount of propanol-2 had been added. Extraction time was not very critical with this solvent system and quite clean gas chromatograms were obtained. Possibly one or two metabolites are co-extracted, but these have not been identified. The procedure is suitable for measuring vinylbital concentrations down to 1 μg/10 ml urine.

CONCLUSION

This procedure for the determination of vinylbital in plasma and urine gives satisfactory results. The method is rapid, specific and sensitive; the extraction time is a critical factor. A calibration curve has to be prepared with each sample series.

REFERENCE

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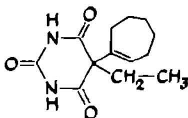
HEPTABARBITAL IN PLASMA AND URINE

INTRODUCTION

Heptabarbital (5-ethyl-5-(1-heptenyl)-barbituric acid), whose structure is shown below, has a relatively low pK_a-value (7.45), and the highest lipophility of the non-N-methylated barbiturates used in the present investigations (Chapter 1 of this Section). It is the only barbiturate with a seven membered ring, that is used in therapy.



heptabarbital

No specific method for the quantitative determination of heptabarbital in biological fluids has been reported previously in the literature. Therefore such a method was developed, using gas chromatography with nitrogen selective detection, for the study of the pharmacokinetics of heptabarbital in man.

MATERIALS AND METHODS

Reagents

Heptabarbital was obtained from CIBA-GEIGY, Arnhem, The Netherlands (a gift of heptabarbital for reference purposes is greatfully acknowledged). Hexobarbital was obtained from OPG, Utrecht, The Netherlands. Diethyl ether, light petroleum, boiling range 40 - 60° and propanol-2 were of analytical-reagent grade (E. Merck). Diethyl ether was freshly distilled before use. Phosphate buffer pH 6.0 was prepared by adding 61.5 ml of a solution con-

taining 6.745 g $Na_2HPO_4 . 12 H_2O$/250 ml of distilled water to 438.5 ml of a solution containing 4.54 g KH_2PO_4/500 ml of distilled water. A standard heptabarbital solution was prepared at a concentration of 1 mg/100 ml absolute ethanol (analytical-reagent grade, E. Merck). A standard hexobarbital solution was prepared at a concentration of 2 mg/100 ml absolute ethanol.

Extraction procedure

Plasma. To 2.0 ml plasma in a conical tube was added 1.0 ml of phosphate buffer pH 6.0 and 0.1 ml of internal standard solution containing 2 μg hexobarbital. The mixture was extracted twice with 5 ml of diethyl ether - light petroleum - propanol-2 (49 : 49 : 2) on a Cenco whirlmixer, for 10 sec twice. The combined organic layers were evaporated to dryness in a stream of dry air. The residue was dissolved in 0.1 ml of absolute ethanol and 2 - 5 μl of this solution were injected into the gas chromatograph.

Urine. The same procedure as for plasma was followed, except that 10.0 ml urine were extracted twice with 10 ml solvent.

Gas chromatography

As described in Chapter 1, Section II.

Preparation of calibration graphs

The concentration of heptabarbital in plasma and urine samples was calculated with the aid of calibration graphs, which were prepared by adding known amounts of heptabarbital to 2.0 ml of blank plasma or 10.0 ml blank urine. The samples were analyzed by the same procedure described above and the ratio of the peak areas of heptabarbital to internal standard was plotted against known concentrations of heptabarbital.

RESULTS AND DISCUSSION

By utilizing the experience gathered with the analysis of vinylbital (Chapter 5 of this Section) the extraction was tried initially with diethyl ether alone, for 3 - 4 sec. A clean gas chromatogram was obtained during the first few minutes, as was shown in the case of vinylbital. Heptabarbital, however, has a realitively long retention time (4.2 min) and it was apparent that there was still slight

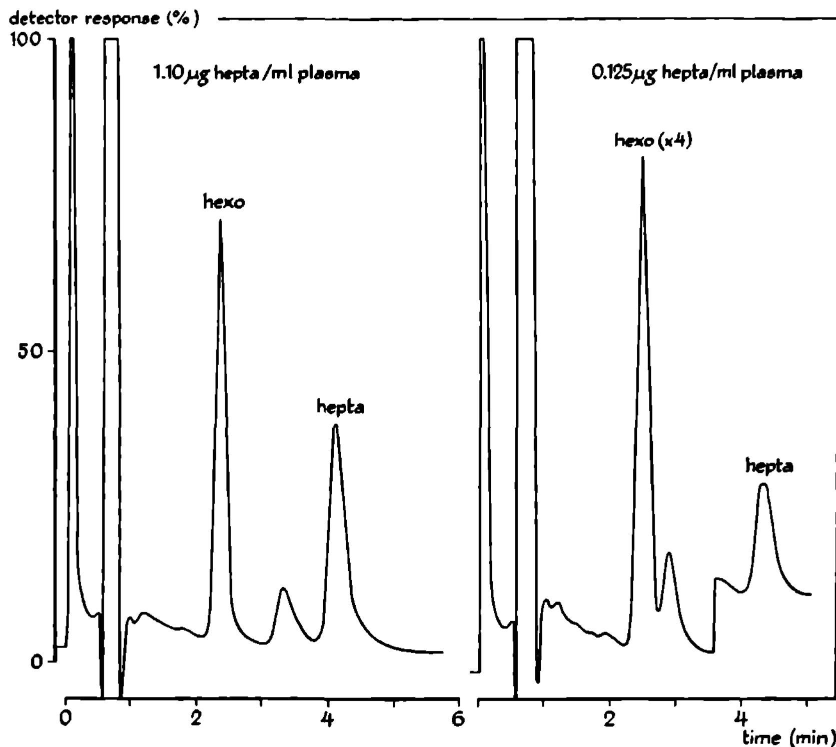


FIG. 1. Gas chromatograms of a 2-ml plasma extract obtained from a subject 6 h after (*left*) and 24 h after (*right*) receiving 200 mg heptabarbital orally. Hexobarbital was used as internal standard (1.0 µg/ml plasma).

interference with concomitantly extracted endogenous plasma material. A mixture of diethyl ether and light petroleum was tried therefore, and this gave satisfactory results, although a small amount of propanol-2 had to be added in order to increase the extraction yield. In Fig. 1 gas chromatograms are shown of plasma extracts containing heptabarbital, with hexobarbital as the internal standard. Once again there is the same plasma peak as noted with the diethyl ether extraction, but there is no interference at 4.2 min. The extraction time is not very critical and 10 sec was chosen subsequently. It is evident that the recovery of heptabarbital from plasma is high (about 92%) (Fig. 2) and constant over a great concentration range. The extraction yield of hexobarbital by this procedure was about 92%.

Calibration graphs (Fig. 3) are linear over a great concentration range. There

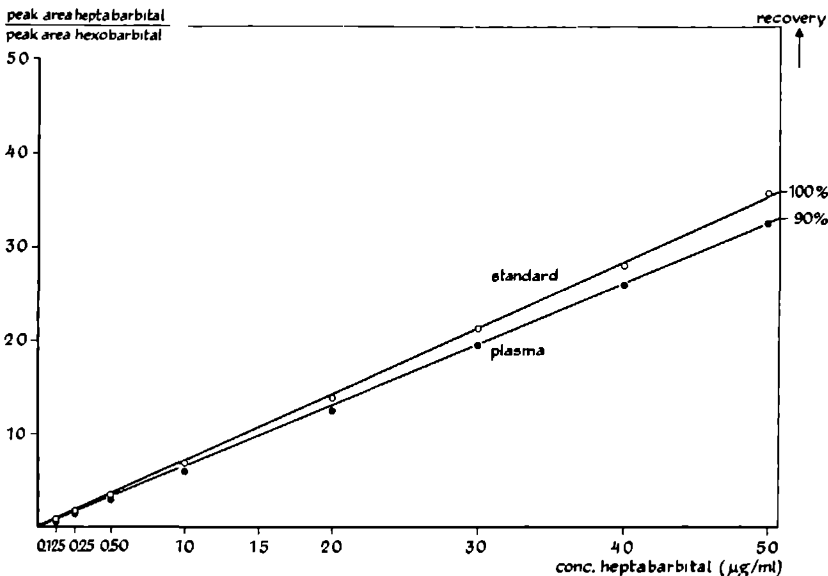


FIG. 2. Peak area ratio of heptabarbital to hexobarbital as a function of known heptabarbital concentrations for the determination of recovery. The plasma curve was obtained in the same way as described for the preparation of a calibration graph, except that 2.0 µg hexobarbital was always added after extraction. The standard curve was obtained by comparing known standard amounts of vinylbital to 2.0 µg hexobarbital.

is some variation with time, but this does not exceed ± 5%. The data are, however, non-randomly distributed around the same mean curve and it is recommendable therefore to construct a calibration graph at regular times.

Equally good results were obtained for the heptabarbital determination in urine. Two extra peaks were present in the gas chromatogram, probably representing heptabarbital metabolites. No attempt has been made yet to identify these compounds.

CONCLUSION

The procedure for the determination of heptabarbital in plasma and urine gives satisfactory results. The method is rapid, specific and sensitive. It is recommended that a calibration curve is prepared with each sample series.

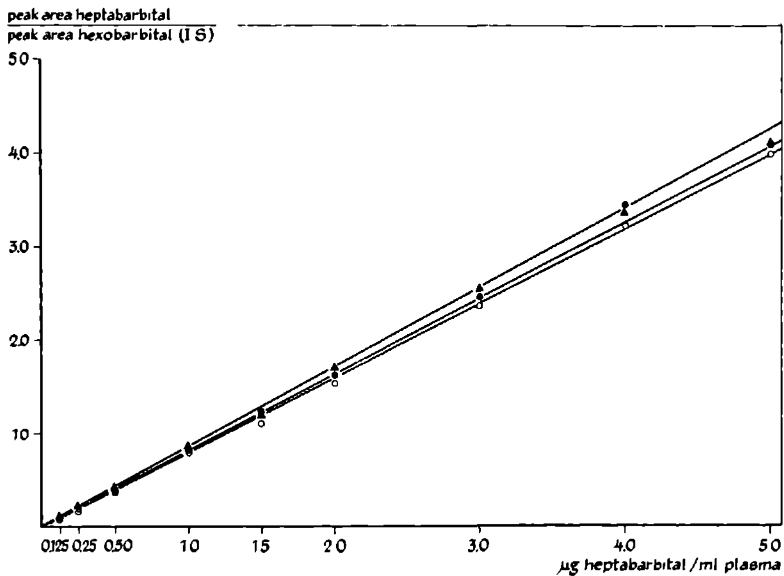
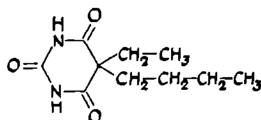


FIG. 3. Calibration graphs for the determination of heptabarbital in plasma, prepared on different occasions, using hexobarbital as internal standard (1.0 μg/ml plasma).

BUTOBARBITAL IN PLASMA

INTRODUCTION

Butobarbital (5-ethyl-5-n-butyl-barbituric acid), whose structure is shown below, is one of the oldest barbiturates used in hypnotic drug therapy, but no pharmacokinetic data are available when therapeutic doses are given to man. Most likely this is due to the lack of a sensitive method for the quantitative determination of the drug in blood or plasma. Therefore such a method was developed, using gas chromatography with nitrogen selective detection.



butobarbital

MATERIALS AND METHODS

Reagents

Butobarbital and hexobarbital were obtained from OPG, Utrecht, The Netherlands. Diethyl ether was of analytical-reagent grade (E. Merck) and was freshly distilled before use. A standard butobarbital solution was prepared at a concentration of 1 mg/100 ml absolute ethanol (analytical-reagent grade, E. Merck). A standard hexobarbital solution was prepared at a concentration of 3 mg/100 ml absolute ethanol.

Extraction procedure

Plasma. To 2.0 ml plasma in a conical tube was added 2.0 ml of distilled water and 0.1 ml of internal standard solution containing 3 μg hexobarbital.

The mixture was extracted twice with 10 ml diethyl ether. Mixing was carried out at half the maximum speed on the whirlmixer (about 20 r.p.s.), twice, during 4 - 5 sec. The combined organic layers were evaporated to dryness in a stream of dry air. The residue was dissolved in 0.1 ml of absolute ethanol and 2 - 5 μl of this solution were injected into the gas chromatograph.

Gas chromatography

As described in Chapter 1, Section II.

Preparation of calibration graphs

The concentration of butobarbital in plasma was calculated with the aid of calibration graphs, which were prepared by adding known amounts of butobarbital to 2.0 ml of blank plasma. The samples were analyzed by the same

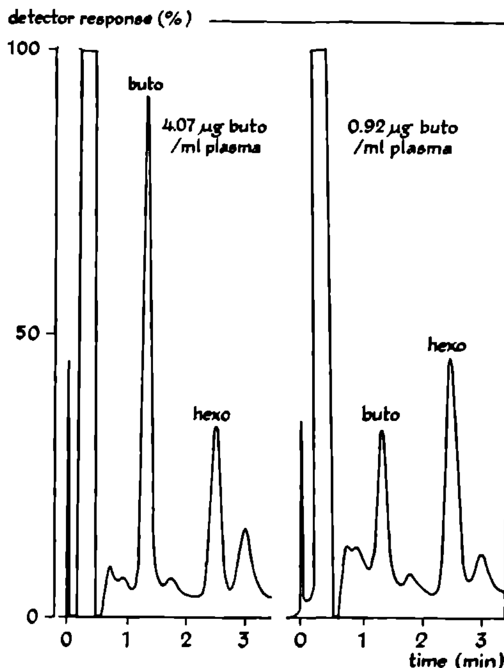


FIG. 1. Gas chromatograms of a 2-ml plasma extract obtained from a subject 1 h after (*left*) and 72 h after (*right*) receiving 200 mg butobarbital orally. Hexobarbital was used as internal standard (1.5 μg/ml plasma).

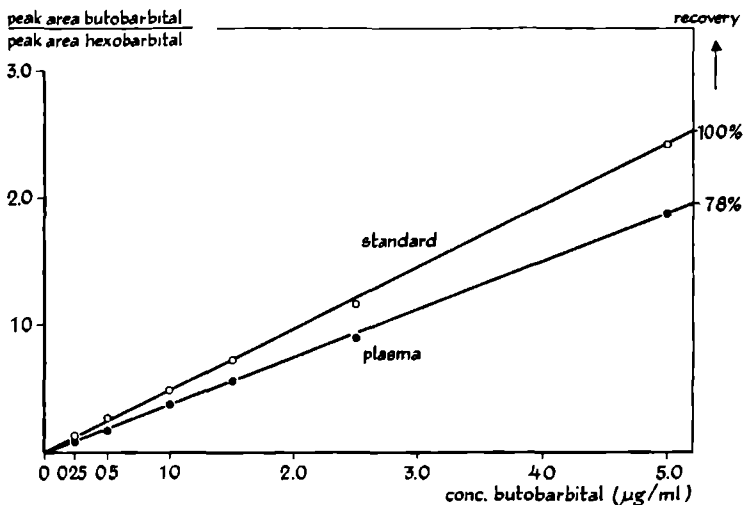


FIG. 2. Peak area ratio of butobarbital to hexobarbital as a function of known butobarbital concentrations for the determination of recovery. The plasma curve was obtained in the same way as described for the preparation of a calibration graph, except that 3.0 µg hexobarbital was always added after extraction. The standard curve was obtained by comparing known standard amounts of butobarbital to 3.0 µg hexobarbital.

procedure described above and the ratio of peak areas of butobarbital to internal standard was plotted against known concentrations of butobarbital.

RESULTS AND DISCUSSION

Special attention was paid to the purity of the butobarbital sample used in the present investigation. De Zeeuw and Wijsbeek (1973) found that in a variety of commercially available batches of butobarbital tablets a second compound was present. They identified this component as 5,5-di-n-butyl-barbituric acid (dibutylbarbituric acid), which is a by-product of the synthesis of butobarbital. In the bulk sample of butobarbital used for the assay and also for the in vivo studies, this compound could not be detected by gas chromatography. Injection of 100 ng of butobarbital sample resulted only in one major peak and it was therefore concluded that the sample did not contain dibutylbarbituric acid in levels higher than 1%.

The extraction procedure of butobarbital from plasma gave no special problems and it is similar to that for vinylbital. Also, the extraction time is again critical and should be restricted to 5 sec. The best results were obtained

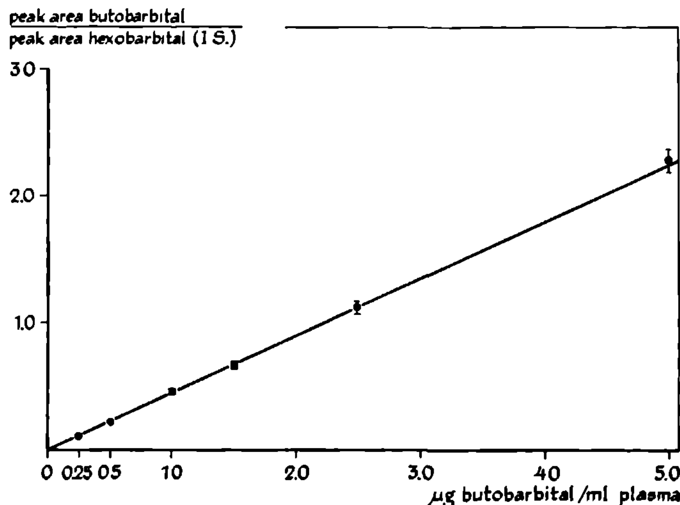


FIG. 3. Calibration graph for the determination of butobarbital in plasma using hexobarbital as internal standard (1.5 μg/ml plasma). Mean values and standard deviations of at least three determinations.

by adding 2.0 ml water to the samples and extracting twice with 10 ml diethyl ether. In Fig. 1 gas chromatograms are shown of a plasma extract containing butobarbital and hexobarbital as the internal standard. Butobarbital has the shortest retention time of all of the barbiturates which were studied. A plasma sample extract is always injected twice into the gas chromatograph, so that a single analysis lasts approximately 6 min. A 78% recovery was found for butobarbital by this procedure (Fig. 2). Concentrations down to 50 ng/ml plasma can be determined. From the calibration curve in Fig. 3, which is composed of at least three individual calibration curves prepared on different occasions, it can be deduced that the procedure is highly reproducible over a large concentration range. Standard deviations did not exceed ± 4%.

CONCLUSION

This method for the assay of butobarbital in plasma is rapid, sensitive and of good precision. It is therefore suitable for the study of butobarbital kinetics in man.

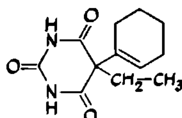
REFERENCE

Zeeuw, R. A. de and J. Wijsbeek (1973), *Pharm. Weekblad 108*, 69.

CYCLOBARBITAL IN PLASMA

INTRODUCTION

Cyclobarbital (5-ethyl-5-(1-cyclohexenyl)-barbituric acid), whose structure is shown below, is structurally related to hexobarbital, since it also contains a cyclohexenyl group. Its lipophility is however much lower, since it is not N-methylated.



cyclobarbital

No specific assay is available for the determination of therapeutic plasma levels in man. Such a method was developed, which requires the preparation of derivatives, using gas chromatography with nitrogen selective detection.

MATERIALS AND METHODS

Reagents

Cyclobarbital was obtained from E. Merck, Darmstadt, G.F.R. and cyclopal (5-ethyl-5-(1-cyclopentenyl)-barbituric acid) was obtained from Siegfried, Switzerland (a gift of this compound for reference purposes is greatfully acknowledged). Diethyl ether, light petroleum, boiling range 40 - 60° and propanol-2 were of analytical-reagent grade (E. Merck). Diethyl ether was freshly distilled before use. Phosphate buffer pH 6.0 was prepared by adding 61.5 ml of a solution containing 6.745 g Na_2HPO_4. 12 H_2O/250 ml of distilled water to 438.5 ml of a solution containing 4.54 g KH_2PO_4/500 ml of distilled water. A standard cyclobarbital solution was prepared at a concentration of 2 mg/100 ml absolute

ethanol (analytical-reagent grade, E. Merck). A standard cyclopal solution was prepared at a concentration of 4 mg/100 ml absolute ethanol. Trimethylanilinium hydroxide (TMAH), about 0.1 M in methanol, was prepared according to the method of Brochmann-Hanssen and Oke (1969).

Extraction and formation of derivatives

Plasma. To 2.0 ml plasma in a conical tube was added 1.0 ml of phosphate buffer pH 6.0 and 0.1 ml of internal standard solution containing 4 μg cyclopal. The mixture was extracted twice with 5 ml of diethyl ether-light petroleum-propanol-2 (49 : 49 : 2) on a Cenco whirlmixer. Mixing was carried out vigorously for about 5 sec (twice). The combined organic layers were evaporated to dryness in a stream of dry air. The residue was dissolved in 50 μl methanol and 25 μl of TMAH solution were added; after homogenization 2 - 5 μl of the mixture were injected into the gas chromatograph.

Gas chromatography

As described in Chapter 1, Section II.

Preparation of calibration graphs

The concentration of cyclobarbital in plasma was calculated with the aid of calibration graphs, which were prepared by adding known amounts of cyclobarbital to 2.0 ml of blank plasma. The samples were analyzed by the extraction and derivatization procedure described above and the ratio of peak areas of cyclobarbital to internal standard was plotted against known concentrations of cyclobarbital.

RESULTS AND DISCUSSION

With the assays of vinylbital, heptabarbital and butobarbital in plasma (Chapter 5, 6 and 7 of this Section), it was observed that a plasma peak with a retention time of 2.5 - 3 min always appeared in the gas chromatogram (see Fig. 1, Chapter 5). This represents endogenous plasma material that was extracted with the common organic solvents. Unfortunately, cyclobarbital coincides with this peak on a 3% OV-17 column. Therefore derivatization of this compound was necessary, in order to change its gas chromatographic behaviour.

Barbiturates may be methylated readily to dimethylbarbiturates, which are

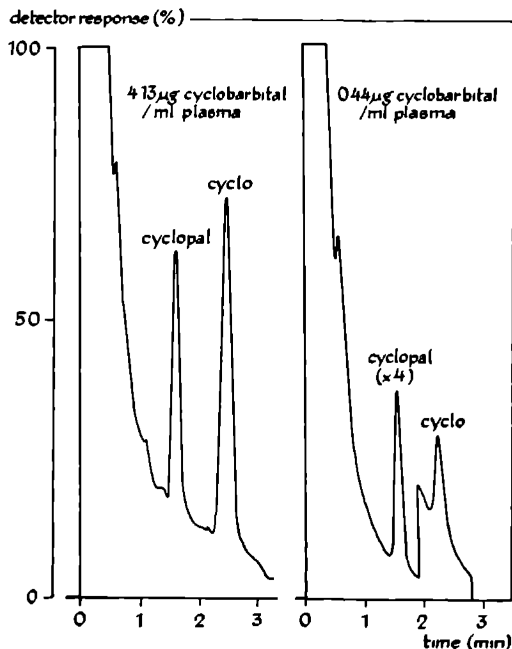


FIG. 1. Gas chromatograms of a 2-ml plasma extract obtained from a subject 7 h after (*left*) and 33 h after (*right*) receiving 300 mg cyclobarbital calcium orally. The peaks in the gas chromatograms represent the dimethylated derivatives of the parent drugs. Cyclopal was used as internal standard (2.0 µg/ml plasma).

more volatile than the unmethylated compounds. Cook et al. (1961) have used diazomethane as a methylating agent, while Martin and Driscoll (1966) preferred to use dimethyl sulfate. Also, Stevenson (1966) reported quantitative on-column methylation with tetramethylammonium hydroxide. The best results were obtained by the procedure of Brochmann-Hanssen and Oke (1969), who improved the procedure by using trimethylanilinium hydroxide, which affords efficient flash-heater methylation. Their method was applied by Ehrnebo et al. (1972) for the plasma assay of amobarbital and pentobarbital and these authors reported highly reproducible results. In the present study also trimethylanilinium hydroxide was used for the determination of cyclobarbital in plasma and the gas chromatograms obtained are shown in Fig. 1. In contrast to non-methylating procedures, a large peak immediately after injection appeared. This is probably due to the elution of the methylating reagent or decomposition products, which contain a nitrogen atom and give rise to a high signal on the nitrogen selective

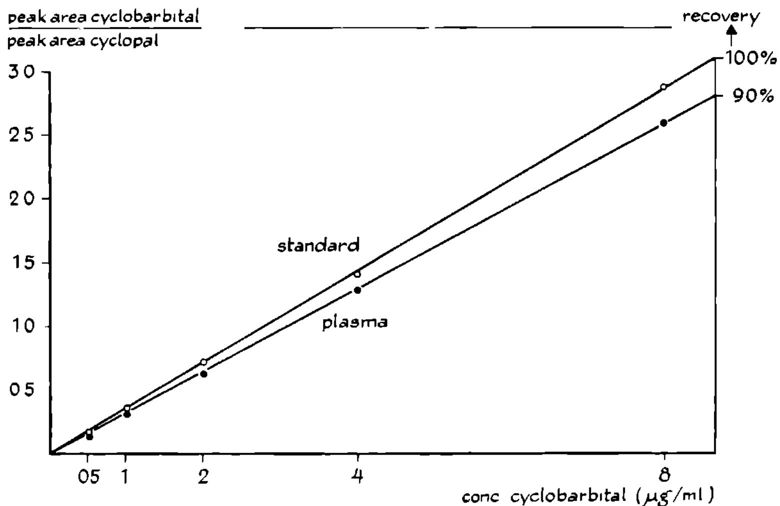


FIG. 2. Peak area ratio of cyclobarbital to cyclopal as a function of known cyclobarbital concentrations for the determination of recovery. The plasma curve was obtained in the same way as described for the preparation of a calibration graph, except that 4.0 μg cyclopal was always added after extraction. The standard curve was obtained by comparing known standard amounts of cyclobarbital to 4.0 μg cyclopal.

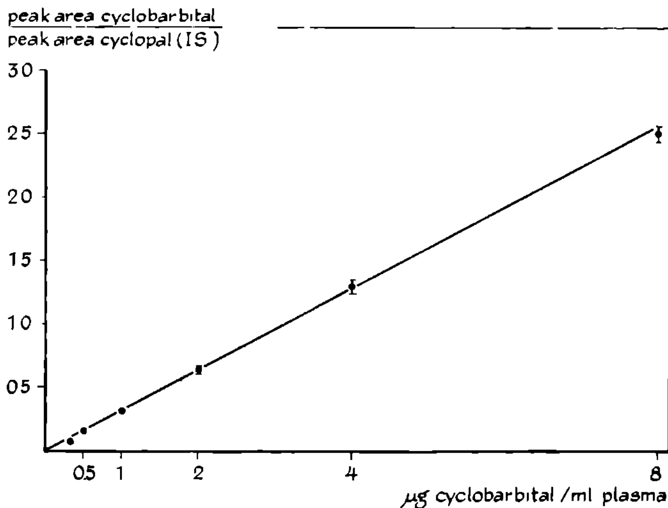


FIG. 3. Calibration graph for the determination of cyclobarbital in plasma using cyclopal as internal standard (2.0 μg/ml plasma). Mean values and standard deviations of at least four determinations.

detector. The compounds to be analyzed, however, are well separated from this injection peak. There was no interference with concomitantly extracted plasma material and concentrations down to 100 ng/ml plasma can be determined in this way. Recovery of cyclobarbital was about 90% when extracting with the mixture of solvents (Fig. 2). From the calibration curve in Fig. 3 for the determination of cyclobarbital in plasma, which is composed of at least four individual curves prepared on different occasions, it can be concluded that the procedure is highly reproducible over a large concentration range. Standard deviations did not exceed ± 5%.

CONCLUSION

This procedure with derivative formation for the quantitative determination of cyclobarbital in plasma yields highly satisfactory results. The method is rapid, specific and sensitive and therefore suitable for the study of cyclobarbital kinetics in man.

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CHLORAL HYDRATE, TRICHLOROETHANOL, TRICHLOROETHANOL-GLUCURONIDE AND TRICHLOROACETIC ACID IN BLOOD AND URINE, EMPLOYING GAS CHROMATOGRAPHY WITH HEAD-SPACE ANALYSIS

INTRODUCTION

The principle metabolites of chloral hydrate (CH) in man are trichloroethanol (TCE), TCE-glucuronide (TCE-Glu) and trichloroacetic acid (TCA), while the CNS depression that follows the ingestion of chloral hydrate is probably due entirely to TCE (Butler, 1948; Marshall and Owens, 1954). Recently, Garrett and Lambert (1973) reported extensively on the pharmacokinetics of TCE and its metabolites in dogs. No detailed studies on the pharmacokinetics of CH and metabolites in humans have been reported so far.

A prerequisite for such studies is a sensitive assay for the simultaneous determination of unchanged drug and metabolites in the same blood or plasma sample. The spectrophotometric methods based on the Fujiwara reaction (Fujiwara, 1916) are in general not specific enough for pharmacokinetic studies after the intake of therapeutic doses in man (Seto and Schultze, 1956; Friedman and Cooper, 1958; Leibman and Hindman, 1964). A specific gas chromatographic method with electron capture detection was described by Garrett and Lambert in 1966 and a modification on this method was reported by the same authors in 1973. However, time-consuming extractions of the rather polar substances into water-immiscible solvents are necessary in this procedure. Direct GC injection of the biological sample has been described for the determination of CH and TCE (Jain et al., 1967), but the risk of contamination of the column and electron capture detector in this technique is substantial.

It was the purpose of the present study to develop a simple, sensitive and specific method for the assay of CH, TCE, TCE-Glu and TCA in blood and in urine. Trichloroacetaldehyde (dehydrated form of CH), TCE and TCA methyl ester are relatively volatile and this property offers the possibility of head-space analysis. When a dissolved substance is sufficiently volatile, the

determination of its concentration in the vapour phase can be used as a measure of the concentration in the liquid phase, provided that an equilibrium between the vapour and liquid phase has been reached. The head-space is the vapour in equilibrium with its liquid phase. With a gas-tight injection syringe, part of the head-space vapour is injected directly into the gas chromatograph. In the literature, several volatile substances have been analyzed in biological samples by applying this principle e.g. inhalation anaesthetics (Yamamura et al., 1966) and ethanol (Bassette and Glendenning, 1968).

In this Chapter the head-space analysis of CH, TCE and TCA is described.

MATERIALS AND METHODS

Materials

Chloral hydrate (OPG, Utrecht, The Netherlands); trichloroethanol (Koch-Light, Colnbrook, Great Britain) was redistilled before use; trichloroacetic acid, p.a. (E. Merck, Darmstadt, G.F.R.); sulphuric acid, 95 - 97%, p.a. (Merck); dimethyl sulphate (BDH, Poole, Great Britain); lead acetate, p.a. (Merck) was used as a 20% solution in water. Sampling bottles were volumetric flasks (25.0 ml), cut at the calibration mark, with self-sealing silicone rubber caps (Beckman, Amsterdam, The Netherlands). Gas-tight Hamilton syringes varying in volume from 100 to 1000 μl were used for taking head-space gas and injection into the gas chromatograph.

Procedure

For the quantitative determination of CH and its metabolites in whole blood, a sample was divided in two portions immediately after it had been taken by vein puncture and a small drop of heparin solution had been added as anticoagulant:

(1) 1.0 ml for the analysis of unconjugated TCE (free TCE); and (2) 2.0 ml for the analysis of free TCE plus conjugated TCE (total TCE), of CH and TCA. These two portions are analyzed as follows.

(1) A 1.0-ml volume of freshly taken whole blood was added to a vial containing 1.0 ml of lead acetate solution. The presence of lead acetate prevents the in vitro conversion of CH into TCE. The contents of the vial were gently homogenized and the vial was then placed in a 60° water-bath, such that the water level was just below the rubber cap, until equilibrium was reached between the liquid and vapour phase (3 h). With a gas-tight syringe, which was maintained at 60° so as to prevent condensation, a known amount of the head-space

vapour was taken and analyzed directly for free TCE by gas chromatography.

(2) A 2.0-ml volume of the same blood sample was added to another vial containing 1.0 ml of concentrated sulphuric acid. The function of this substance is outlined under Results and Discussion. After homogenizing the sample, the vial was equilibrated at 60° for 3 h. A known amount of the head-space vapour was taken and analyzed for CH and total TCE. The TCE-Glu concentration was calculated from the difference between the total TCE and free TCE. Then 0.10 ml of dimethyl sulphate was added by means of a syringe in order to convert the TCA into its methyl ester. After re-equilibration for 4 h, the head-space vapour was analyzed for this compound.

The procedure for the analysis of urine samples is exactly the same as that for whole blood, except that the lead acetate solution is omitted. When relatively high concentrations were encountered, the urine samples were diluted with distilled water until proper measurement was possible.

The concentration of CH and metabolites in the samples was calculated with the aid of calibration graphs, prepared by adding known amounts dissolved in a maximum of 0.1 ml of distilled water to blank samples of blood or urine. These samples were run through the procedure as described. The peak height per 1000 μl of head-space vapour was plotted against known concentrations of the compounds. The detector response was calibrated daily by injection of a known amount of standard solution of TCE in ethanol (0.10 mg per 100 ml).

Gas chromatography

A Hewlett-Packard gas chromatograph, Model 402, with a Hewlett-Packard nickel-63 electron capture detector, Model 2-6195, and equipped with a Hewlett-Packard strip-chart recorder, Model 7127A, was used. Glass columns (1.8 m x 3 mm I.D.) were packed with 10% OV-17 on Gas-Chrom Q, 80 - 100 mesh (Applied Science Labs., State College, Pa., U.S.A.). The injector block was maintained at 150°, the column at 125° and the electron capture detector at 200°. Flow-rates were 20 ml/min for the carrier gas (nitrogen) and 60 ml/min for the purge gas (argon-methane, 95 : 5). The electron capture detector was operated at pulse 5 and the electrometer setting was kept continuously at range 10, attenuation 8.

RESULTS AND DISCUSSION

A major advantage of head-space analysis is that no time-consuming extraction procedure is required before analysis can be carried out. This advantage

is particularly important for the relatively polar substances studied in this work, because they are difficult to extract into water-immiscible solvents. Garrett and Lambert (1966; 1973) used diethyl ether, for which the following partition coefficients were determined: CH 0.191 and TCE 22. No partition coefficient is available for TCA, but it seems likely that it is very unfavourable with respect to the organic phase. Therefore, only TCE appears to have sufficient lipophility for a substantial extraction yield. Nevertheless, Garrett and Lambert seemed to obtain satisfactory results for all compounds with extraction by diethyl ether. The other gas chromatographic method that has been described for the analysis of CH and its metabolites is the technique of direct injection of the biological sample (Jain et al., 1967). However, it has been our experience that direct injection of aqueous solutions results in poor peak reproducibility with electron capture detection.

It is the relative volatility of trichloroacetaldehyde, TCE and TCA methyl ester that offers the possibility of head-space analysis, although a relatively

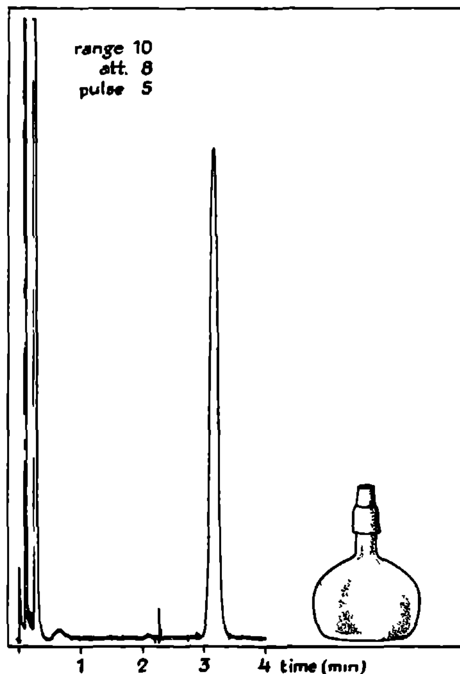

FIG. 1. *Left:* Gas chromatogram of trichloroethanol after direct injection of head-space gas, in equilibrium with a 1.0-ml blood sample + 1.0 ml of lead acetate solution. The TCE blood concentration was 8.0 μg/ml. *Right:* Equilibration flask with rubber cap.

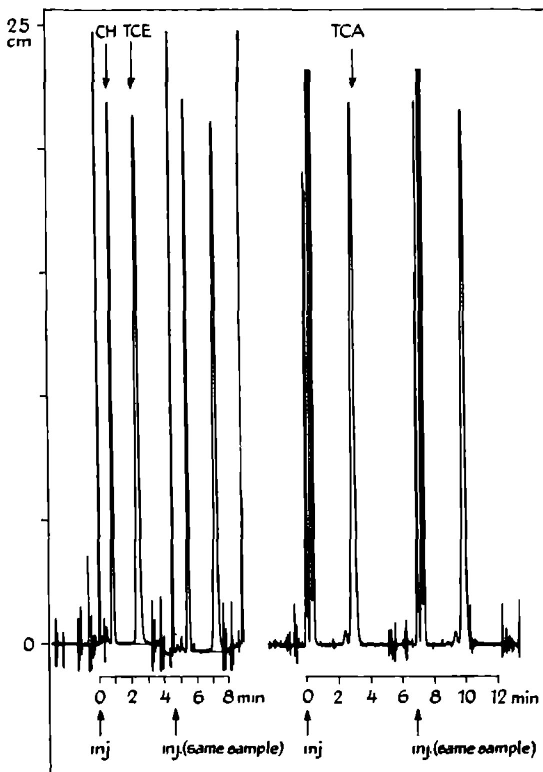

FIG. 2. Gas chromatograms of chloral hydrate (CH), trichloroethanol (TCE) and trichloroacetic acid methyl ester (TCA) after direct injection of head-space gas, in equilibrium with a 2.0-ml blood sample + 1.0 ml of concentrated sulphuric acid. Blood concentrations of the compounds were: CH, 10.2 μg/ml; TCE, 12.6 μg/ml (injection volume 1000 μl) and TCA, 10.5 μg/ml (injection volume 500 μl).

high equilibration temperature and a long equilibration time are required. In Fig. 1 and 2 typical gas chromatograms are shown after direct injection of the head-space gas in equilibrium with a blood sample containing CH, TCE and TCA. The times after which equilibrium was reached between the concentration in the liquid phase and in the head-space were verified by analyzing five standard solutions of CH, TCE and TCA at several time intervals. In Fig. 3, the curves obtained for TCE and TCA are shown and it can be concluded that TCE reaches equilibrium after about 3 h and TCA after about 4 h. Also, a time of 3 h was found for CH. The relatively high temperature of 60° has been chosen arbitrarily; it results in an enrichment of the head-space vapour

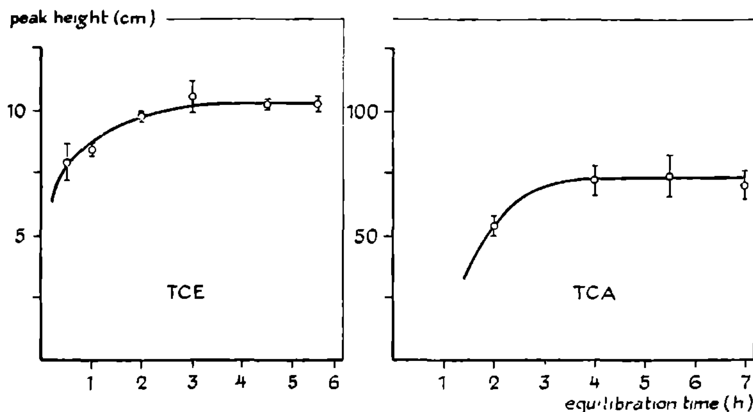

FIG. 3. Change of concentration (peak height per 1000 μl of head-space gas injected) of TCE and TCA methyl ester in the gas phase during equilibration at 60° (2.0 ml of blood + 1.0 ml of concentrated sulphuric acid). Concentration of TCE, 5.0 μg/ml blood (injection volume 1000 μl) and concentration of TCA, 18.0 μg/ml blood (injection volume 250 μl). Mean values ± standard deviation for five determinations.

and it was observed that shorter equilibrium times were required than at lower temperatures.

When using the column packings described in the literature, CH and TCE exhibit relatively broad peaks. Therefore, several stationary phases were investigated, including OV-17, OV-17 plus orthophosphoric acid, Carbowax 20M and Carbowax 20M-terephthalic acid. A loading of 10% OV-17 on Gas-Chrom Q (80 - 100 mesh) proved to be the most satisfactory packing. A good separation was obtained and for all compounds symmetrical and well defined narrow peaks with short retention times were obtained (Fig. 2). The repeated injection of the same sample demonstrates the good reproducibility (standard deviation ± 3%). The gas chromatogram of a blank blood or urine sample treated in the above manner is completely clean and there are no interfering peaks due to normal blood or urine constituents (Fig. 1).

It seems obvious that for the quantitative determination of this type of compounds, an electron capture detector should be used. The response of a normal flame ionization detector is determined by the number of C-H bonds per molecule and these are few in CH and its derivatives. However, the response of an electron capture detector depends on the electron affinity of the compound to be analyzed, and this is high for halogen-containing substances. Garrett and Lambert (1966; 1973) found that the sensitivity of the flame ionization detector

is only slightly greater than the Fujiwara UV method according to Friedman and Cooper (1958), while their own method with electron capture detection was much more sensitive. In the present study, use was made of a nickel-63 electron capture detector with 0.75-μsec voltage pulses and intervals of 5, 15, 50 and 150 μsec. Although the sensitivity increases with increasing pulse interval, the sensitivity obtained with the lowest pulse interval is satisfactory for physiologically encountered drug concentrations. A practical disadvantage of the electron capture detector is its small linear dynamic range. However, this gave no problems when using a pulse interval of 5 and electrometer settings of range 10 and attenuation 8. Every response smaller than full-scale deflection (25 cm) proved to be out of the linear dynamic range (Fig. 4). If this was not the case, the injection volume was reduced. The quantitative parameter used in this study was the peak height per 1000 μl of head-space vapour. That there is no essential difference in choosing the peak height or peak area as a measure for the detector response can also be concluded from the results shown in Fig. 4.

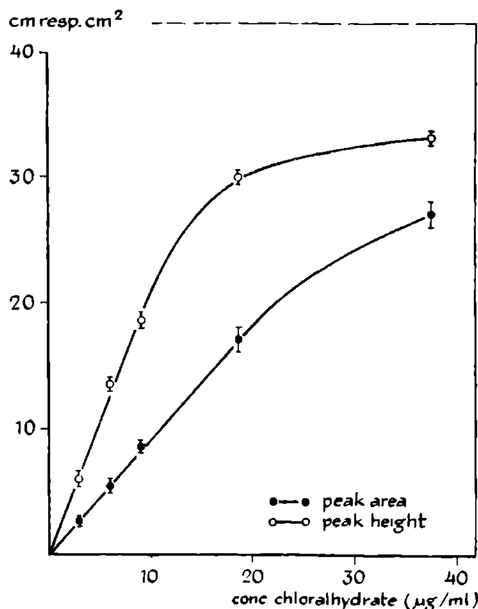

FIG. 4. Calibration curves for CH in blood. For each concentration, the injection volume was 1000 μl of head-space gas. Note the linearity over the first 25 cm, which corresponds to full scale deflection of the recorder at range 10, attenuation 8 (electron capture pulse 5). Also, there is no essential difference between choosing peak height or peak area as a measure of the detector response. Mean values $\pm$ standard deviation for five determinations.

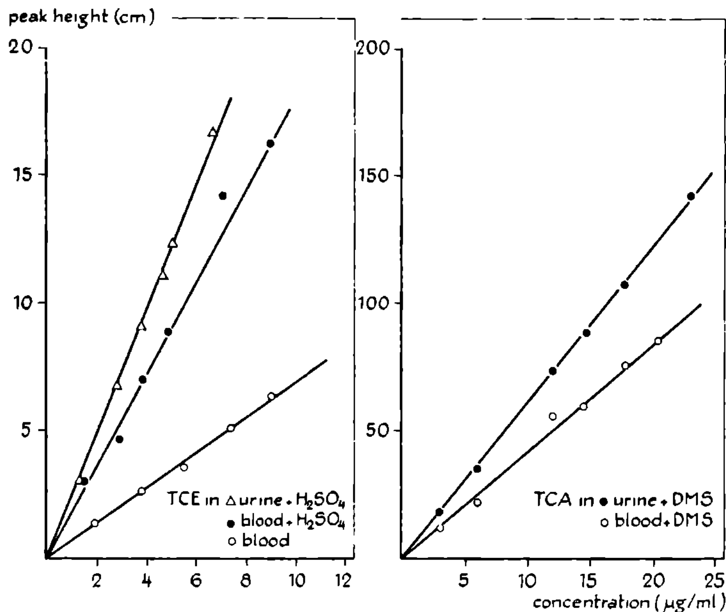

FIG. 5. Calibration curves for TCE, TCE in the presence of sulphuric acid and TCA in blood and in urine. The detector response was measured as the peak height per 1000 μl of head-space gas. The volumes injected were such that the detector response was in the linear dynamic range.

Calibration curves for the different compounds obtained by the procedure described are shown in Fig. 5. There is a linear relationship between detector response and concentration, provided that the injection volume has been adapted to the linear dynamic range of the detector. The sensitivity, however, is dependent on the composition of the liquid phase, blood or urine and the compound in question. Also, sulphuric acid has a great influence on the concentration of the three substances in the head-space gas. It enhances the formation of trichloroacetaldehyde, which is the more volatile form of CH. The increase in response of TCA methyl ester is about a factor 10 in the presence of sulphuric acid. The primary reason, however, for adding this strong acid to the samples is to achieve hydrolysis of TCE-Glu into TCE, so that total TCE can be determined and then conjugated TCE can be calculated by difference. Sulphuric acid also prevents the in vitro conversion of CH into TCE, as does lead acetate. This is necessary, for Butler (1949) reported on the very rapid in vitro reduction of CH by erythrocytes. Therefore, it is important that immediately after they have

been taken, the blood samples should be mixed with sulphuric acid or lead acetate. Analysis may then follow within 48 h.

There is also the possibility of determining plasma concentrations instead of blood concentrations, in which case the blood has to be centrifuged immediately after the sample has been taken.

For the esterification of TCA, dimethyl sulphate is used, and as this is an equilibrium reaction it could be expected that the amount of dimethyl sulphate added influences the amount of ester that is formed. It was observed that the addition of 0.30 ml instead of 0.10 ml of dimethyl sulphate results in an increase in the response by a factor of about 2.2. This effect means that the amount of dimethyl sulphate must be carefully added by means of a calibrated syringe. At first, it was tried to determine CH, TCE-total and TCA simultaneously, as there is a good gas chromatographic separation of the three compounds. However, dimethyl sulphate appeared to influence the response of TCE in an irreproducible manner and therefore TCE has to be determined in the absence of dimethyl sulphate. This requires a separate equilibration period for the analysis of TCA.

The detection limits, under the conditions applied, are ca. 0.5 μg/ml for CH and TCE and 0.1 μg/ml for TCA in blood or urine. Lower detection limits can easily be obtained by increasing the pulse interval. The precision was determined by measuring the peak height after adding known concentrations of the compounds to a blank sample of blood or urine. Standard deviations did not exceed $\pm$ 6% (five determinations), except for free TCE, for which the standard deviation was 6 - 8% (five determinations).

CONCLUSION

The method described for the determination of chloral hydrate and metabolites in blood and urine is simple and sensitive, and the precision is satisfactory. It is therefore suitable for the study of pharmacokinetics and metabolism of chloral hydrate in man.

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SECTION III

PHARMACOKINETICS AND BIOPHARMACEUTICS OF BARBITURATES AND CHLORAL HYDRATE

CHAPTER 1

HEXOBARBITAL AND HEXOBARBITAL SODIUM

A. GENERAL INTRODUCTION
B. INTRAVENOUS ADMINISTRATION
C. ORAL AND RECTAL ADMINISTRATION

A. GENERAL INTRODUCTION

Hexobarbital was introduced into drug therapy in 1931. Its sodium salt has been used widely as an anaesthetic agent which is administered intravenously for the production of complete anaesthesia of short duration or for the induction of general anaesthesia. This application is similar to that of thiopental sodium, but hexobarbital has been largely superseded by thiopental, methohexital and non-barbiturate intravenous anaesthetics (Price and Dripps, 1970). The description „very short-acting" or „ultrashort-acting", which is often associated with the anaesthetic barbiturates (Sharpless, 1970; Martindale, 1972), is misleading and this has been referred to by Mark (1969). These compounds are not rapidly inactivated in the body, but the prompt recovery from anaesthesia following small doses, is due primarily to redistribution of the active substances from the brain to other less well-perfused tissues (Mark et al., 1949; Richards and Taylor, 1956; Price et al., 1960). Higher doses may result in CNS depression of a very long duration (Tatum, 1939; Brodie et al., 1951; Brodie, 1952). The pharmacokinetic behaviour of many of these drugs, including hexobarbital, is not elucidated yet. The reason that hexobarbital can be used as an anaesthetic agent may be explained on the basis of its moderately high lipid solubility, as evidenced by its partition coefficient compared to non-N-methylated barbiturates (Chapter 1, Section II). It has been shown previously for barbiturates, that a high lipid solubility favours rapid brain penetration (Brodie et al., 1960; Mark, 1963). Although the lipophility of hexobarbital is sufficiently high to permit an instantaneous passage through the blood-brain barrier,

it is lower than some other barbiturates used for i.v. anaesthesia, including thiopential and methohexital (Bush et al., 1966). This may explain the prevailing opinion that the duration of hexobarbital CNS depressant action is a reflection primarily of metabolism rather than of redistribution from adipose tissue, as has been shown for thiopental (Richards and Taylor, 1956; Price et al., 1960; Bush and Weller, 1972). This explains why hexobarbital has been used as a model substrate in hundreds of studies concerning the activity of the mixed - function oxygenase system in the liver, where the compound is metabolized (Cooper and Brodie, 1955; Toki et al., 1963). Its hypnotic (anaesthetic) action is such that suitable and convenient doses, injected i.p. to rats or mice, produce a profound sleep of moderate duration. Thus, either shortening or lengthening of the sleeping time can be measured with acceptable accuracy (Stitzel et al., 1966; Bush and Weller, 1972). The change in sleeping time is then considered as a measure for the change of oxidative enzyme activity.

Bush and Weller (1972) have reported an extensive review of the fate of hexobarbital in different species. In man information is apparently very scarce. Brodie (1952) has measured blood levels after i.v. administration of 3 g hexobarbital sodium to one human subject and it has been deduced, from the blood concentration curve, that the half-life of this drug was about 5 h. However, it should be realized that the administered dose was exceptionally high. Blood levels after oral administration of hexobarbital sodium to human volunteers were reported by Bush et al. (1966) and by Sjögren et al. (1965). Concentrations, however, could only be followed for a few hours, so that the estimation of half-lives was impracticable. It is evident therefore, that even though hexobarbital is a drug that has been in use for many years, its pharmacokinetics have not been well defined. Improvement in the sensitivity of the assay for hexobarbital (Breimer and van Rossum, 1974; Chapter 2, Section II) makes it feasible to determine the pharmacokinetics after therapeutic doses to man.

The primary reason that hexobarbital and hexobarbital sodium were included in the present investigation on the pharmacokinetics of hypnotic drugs in man, was based upon the impression that hexobarbital was rapidly metabolized in man (Brodie, 1952). Alvarez (1971) showed that this compound was the barbiturate that was most rapidly metabolized in rats, when compared with several other barbiturates. A further advantage may be the fact that its duration of action is not complicated by a strong accumulation into fat tissue (Richards and Taylor, 1957). In a clinical trial on the effectiveness of several barbiturates in insomnia, hexobarbital (as free acid in a 500 mg dose) was shown to be moderately effective. Bush et al. (1966) showed that the sodium salt of some anaesthetic barbiturates, including hexobarbital, did produce rapid and intense

sedation with relative short duration. These and other authors (Way and Trevor, 1971) concluded that the sodium salt of thiopental or hexobarbital may be desirable for patients who have difficulties in getting to sleep. If the half-life of the drug is relatively short, natural sleep may predominate again shortly after falling asleep. On the other hand, hexobarbital as free acid may be used for maintaining sleep, since the absorption rate of this form is probably slower (Sjögren, 1965).

Firstly, the pharmacokinetics of hexobarbital were studied following intravenous administration of the drug to healthy human volunteers. The advantage of i.v. administration is that bioavailability uncertainty does not exist by definition, so that reliable estimation of parameters is possible. Moreover, these healthy volunteers serve as controls in the study on the pharmacokinetics of hexobarbital in patients with liver disease (see Section IV).

Secondly, several pharmaceutical formulations of hexobarbital and hexobarbital sodium were studied, mainly with respect to their absorption rate. This will be discussed for a comparison of the usefulness of a certain preparation in hypnotic drug therapy with hexobarbital. The administered dose was usually 600 mg, because it appeared in preliminary studies that following this dose the plasma concentration could be measured for sufficiently long periods of time. Furthermore the results of Hinton (1963) have indicated that a 500 mg dose of free acid is only moderately effective.

Finally, plasma levels during repetitive hexobarbital administration were measured in order to determine if accumulation of the drug in the body would occur and also to ascertain whether the half-life of the drug would remain unchanged.

B. INTRAVENOUS ADMINISTRATION

METHODS

Hexobarbital sodium (EVIPAN) for i.v. injection was obtained from BAYER AG, Leverküsen, GFR. Solutions were prepared by dissolving, immediately before use, the sterile contents of an ampoule in sterile water (100 mg/ml).

Fourteen healthy male volunteers, ranging in age from 20 - 25 years and in body-weight from 50 - 83 kg, participated in the study. They had received no regular medication during the 4 weeks preceding initiation of the experiments. After an overnight fast, at 9 a.m., the subjects received hexobarbital sodium intravenously by 30 min or 60 min zero-order infusion. The doses applied

(calculated as hexobarbital free acid), were in the range of 7.23 - 8.82 mg/kg (see Table I). A relatively slow infusion procedure for i.v. drug administration was preferred in order not to discomfort the volunteers and to avoid systemic reactions which often follow rapid i.v. administration anaesthetic barbiturates (Price and Dripps, 1970). The volunteers were kept lying for about 3 h after drug infusion. Blood samples (5 ml) were taken from a forearm vein at the following times during a 30 min infusion: 5, 10, 15, 20, 25 and 30 min and at 10, 20, 30, 40, 50 and 60 min in the case of a 60 min infusion. Furthermore blood samples were obtained usually at 10, 20, 30 min and 1, 2, 4, 6, 8, 10, 12, 18 and 24 h post infusion. The blood was heparinized, centrifuged and the plasma was separated and frozen until assayed. The hexobarbital plasma concentrations were determined by gas chromatography with nitrogen selective detection, as described in Chapter 2, Section II. Five subjects collected their 24 hours' urine after hexobarbital administration and the amount of unchanged drug and 3-keto-hexobarbital was determined as described in Chapter 2 and 3 respectively, Section II.

RESULTS

During i.v. infusion of hexobarbital sodium a rapid rise in the hexobarbital plasma concentration occurred until the zero-order infusion was terminated. After this event the plasma concentration-time curve exhibited two distinct phases; an early phase with a relatively steep slope and a latter phase with a more gradual slope (Fig. 1). This suggests that the early rapid drop in hexobarbital plasma concentration is dictated by rapid tissue localization, whereas the subsequent more gradual slope is a reflection of the elimination of the drug. This kinetic behaviour is consistent with the conception that for hexobarbital the body consists of two kinetically distinct compartments: a central compartment and a peripheral compartment (Riegelman et al., 1968). The post-infusion plasma concentration curve can be represented mathematically as a sum of two exponentials according to Loo and Riegelman (1970):

$$C_p = A_1^* . e^{-t^*/\tau_1} + A_2^* . e^{-t^*/\tau_2} \qquad \text{(Eq. 1)}$$

where C_p is the post-infusion plasma concentration A_1^* and A_2^* are the coefficients (concentration intercepts of the exponential terms at $t^* = 0$), τ_1 and τ_2 are the time constants and t^* is the time after the end of the infusion i.e. $t^* = t - T$, where T is the infusion time.

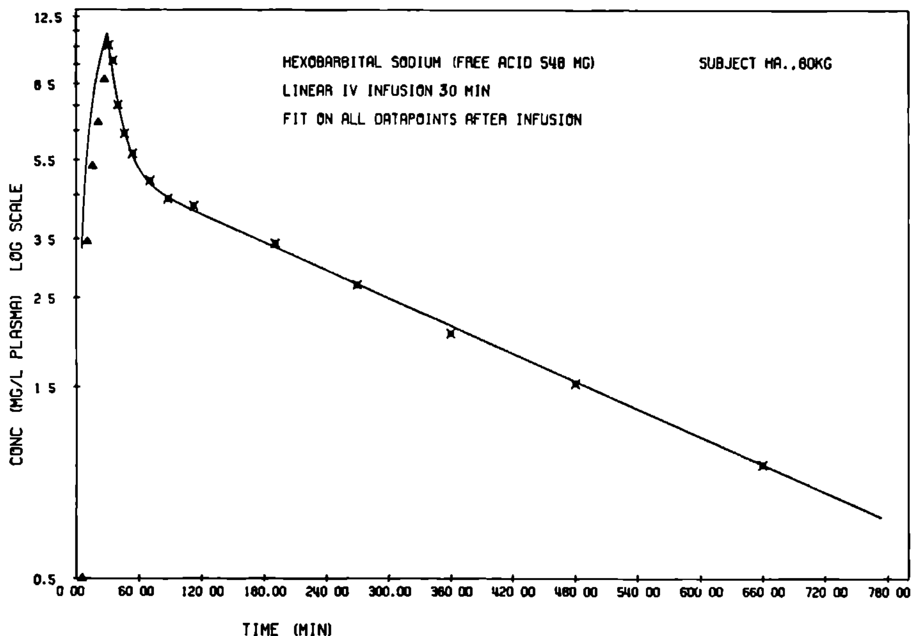

FIG. 1. Hexobarbital plasma concentration curve on semi-logarithmic scale during and after a 30 min zero-order i.v. infusion of hexobarbital sodium into a healthy volunteer. The post-infusion concentrations were fitted according to Eq. 5. The curve during infusion was calculated on basis of the parameters of the post-infusion curve; the concentrations during this phase (triangles) have not been used in the fitting procedure. The picture has been taken from a direct computer plot.

During infusion the plasma concentration, based on two compartment kinetics, can be described by the following equation:

$$C_p = A_1 \cdot \frac{\tau_1}{T}(1 - e^{-t/\tau_1}) + A_2 \cdot \frac{\tau_2}{T}(1 - e^{-t/\tau_2}) \qquad \text{(Eq. 2)}$$

where A_1 and A_2 are the hypothetical intercepts with the ordinate for an intravenous bolus injection of the same amount of drug (i.e. $T = 0$). It is evident that A_1 and A_2 are equal to $A_1{}^*$ and $A_2{}^*$ if $T = 0$, but the differences increase as the infusion time increases. The relationship between $A_1{}^*$, A_1, and $A_2{}^*$, and A_2 is shown by the following equations (Loo and Riegelman, 1968):

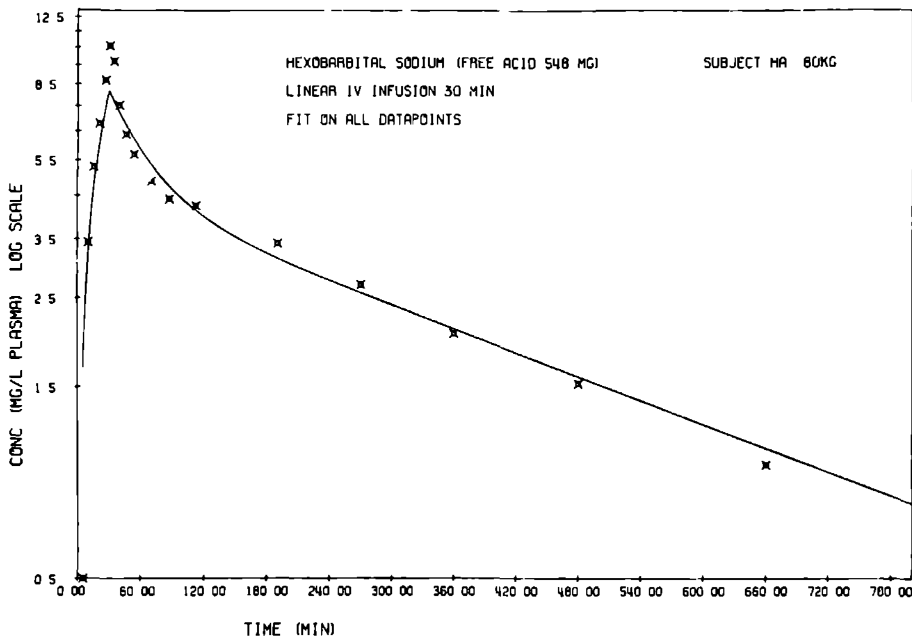

FIG. 2. The same experiment as in Fig. 1, but now concentrations during infusion were included in the fitting procedure, according to Eq. 6. It can be noted that there is now less agreement between the experimental data and the computed curve than in the case of Fig. 1 The picture has been taken from a direct computer plot.

$$A_1^* = A_1 \cdot \frac{T}{\tau_1}(1 - e^{-T/\tau_1}) \qquad \text{(Eq. 3)}$$

$$A_2^* = A_2 \cdot \frac{T}{\tau_2}(1 - e^{-T/\tau_2}) \qquad \text{(Eq. 4)}$$

Once the hypothetical intercepts A_1 and A_2 together with the time constants have been determined, the pharmacokinetic parameters intrinsic to a two-compartment open model can be calculated.

In the present investigation with hexobarbital, use was made of the FARM-FIT computer program and the post-infusion plasma concentrations were fitted according to the following general equation:

$$C_p = \sum_{i=1}^{n} A_i \cdot \frac{\tau_i}{T}(1 - e^{-T/\tau_i}) \cdot e^{(T-t)/\tau_i} \qquad \text{(Eq. 5)}$$

A two-term exponential is assumed to describe the curve adequately (two-compartment model, n = 2). The initial graphical estimates of τ_1 and τ_2 were used as a start guess. A relative error of 5% in the plasma concentration was taken into account, since this is the standard deviation in the assay procedure (Chapter 2, Section II). An example of a fitted and directly plotted curve is given in Fig. 1 and it illustrates the agreement between the plasma levels and the two-compartment open model. The curve that was obtained during the infusion period, was simulated on the basis of the post-infusion parameters. It may be noticed that the experimental data, obtained for this subject during this period, are somewhat lower than theoretically expected. Initially these data were included for the computation of the best fitted total curve, which occurred according to the following general equation, and is valid during infusion:

$$C_p = \sum_{i=1}^{n} A_i \cdot \frac{\tau_i}{T}(1 - e^{-t/\tau_i}) \qquad \text{(Eq. 6)}$$

A two-term exponential was supposed to underly the curve during infusion. The results of the combined fitting according to Eq. 5 and Eq. 6 are given in Fig. 2, for the same experiment as in Fig. 1. It can be observed now that there is less agreement between the experimental data and the computed curve, which also became apparent in the higher estimated standard errors of the fitted parameters. Although it is unlikely that the kinetic behaviour of hexobarbital during infusion is fundamentally different from the behaviour after infusion, it may be expected that the plasma concentration during the relatively rapid infusion period does not always follow two-compartment kinetics exactly. If these data are nevertheless included in the fitting procedure for the two-compartment model, relatively large errors may be induced. It was therefore decided to use concentrations after infusion only, to obtain the more accurate model parameters.

In Table I the pharmacokinetic parameters of hexobarbital in each of the fourteen volunteers as well as the mean values, are given. All the values for each individual are reported since it is considered important to present the degree of variability of the kinetics of a drug in man and also to examine the validity of the model for each individual. For the fitted parameters (τ_1, τ_2, A_1 and A_2) the estimated standard errors are given and it is evident that these are usually substantial with respect to τ_1 and A_1. These parameters describe the

TABLE I. Pharmacokinetic parameters of hexobarbital after intravenous administration to fourteen healthy male volunteers, according to the two-compartment open model (estimated standard errors of fitted parameters in parentheses).

Name	K.H.		H.R.		B.W.		E.R.		K.B.	
Age (yr)	22		23		23		24		25	
Body-weight (kg)	73		80		80		74		63	
Dose (free acid; mg)	548		548		548		548		461	
Dose/kg (mg)	7.51		6.85		6.85		7.41		7.32	
Inf.time (min)	60		30		60		30		60	
τ_1 (min)	15.1	(43.1%)	11.7	(8.4%)	8.6	(39.0%)	5.95	(19.2%)	22.8	(8.4%)
τ_2 (min)	366	(7.4%)	369	(2.1%)	290	(6.2%)	365	(5.5%)	230	(3.3%)
$t_{1/2}$ (min)	254		256		201		253		160	
A_1 (mg/l)	13.7	(51.3%)	17.1	(10.1%)	30.2	(54.1%)	54.4	(30.3%)	8.70	(9.4%)
A_2 (mg/l)	4.94	(8.0%)	5.41	(2.0%)	4.94	(7.1%)	5.63	(4.5%)	5.30	(3.0%)
V_1 (l)	29.5		24.3		15.6		9.13		32.9	
V_1/kg (l)	0.40		0.30		0.20		0.12		0.52	
V_f (l)	89.8		83.9		80.0		72.9		65.3	
V_f/kg (l)	1.23		1.05		1.00		0.99		1.03	
k_{el} (ml/min)	272.2		249.5		323.9		230.5		326	
k_{el}/kg (ml/min)	3.73		3.12		4.05		3.11		5.17	
r_{12} (1/min)	0.040		0.055		0.080		0.127		0.019	
r_{21} (1/min)	0.020		0.022		0.019		0.018		0.019	
k_{12} (ml/min)	1180		1340		1247		1162		627	

TABLE I continued.

Name	B.E.	F.B.	M.K.	B.B.	D.A.
Age	23	25	24	22	24
B-W	69	77	70	73	69
Dose	505.1	563.5	512.5	534.4	505.1
D/kg	7.32	7.32	7.32	7.32	7.32
T	60	60	60	60	60
τ_1	31.0 (37.2%)	19.6 (31.9%)	28.4 (32.6%)	33.5 (49.2%)	28.2 (25.9%)
τ_2	441 (6.1%)	343 (6.6%)	389 (4.6%)	368 (6.4%)	299 (9.3%)
$t_{1/2}$	306	238	270	256	208
A_1	9.05 (16.8%)	18.1 (40.8%)	9.95 (31.1%)	9.96 (49.3%)	17.0 (28.6%)
A_2	6.20 (10.2%)	3.90 (11.3%)	5.56 (8.0%)	4.20 (13.6%)	3.86 (16.3%)
V_1	33.1	25.6	33.0	37.7	24.2
V_1/kg	0.48	0.33	0.47	0.52	0.35
V_f	67.5	91.6	72.7	87.8	67.9
V_f/kg	0.98	1.19	1.03	1.20	0.98
k_{el}	167.5	332.6	209.2	284.5	308.8
k_{el}/kg	2.43	4.32	2.99	3.90	4.48
r_{12}	0.015	0.030	0.017	0.014	0.017
r_{21}	0.014	0.011	0.014	0.011	0.009
k_{12}	497	755	566	539	405

TABLE I continued.

Name	S.G.		W.G.		P.F.		S.M.		Mean	± S.D.
Age	22		23		20		21		22.9	± 1.4
B-W	50		80		83		68		71.6	± 8.2
Dose	366.0		585.6		600		600		530.4	± 61.1
D/kg	7.32		7.32		7.23		8.82		7.37	± 0.46
T	60		60		60		60			–
τ_1	81.6	(21.4%)	42.6	(19.5%)	39.6	(35.0%)	48.7	(35.1%)	29.8	± 19.7
τ_2	505	(4.0%)	635	(6.6%)	304	(8.7%)	366	(8,2%)	376	± 99
$t_{1/2}$	351		441		211		254		261	± 69
A_1	6.79	(11.4%)	13.2	(18.8%)	6.52	(23.9%)	6.70	(19.5%)		–
A_2	4.79	(7.9%)	3.33	(8.6%)	4.64	(12.9%)	6.82	(10 8%)		–
V_1	31.6		35.5		53.8		44.4		30.7	± 11.1
V_1/kg	0.63		0.44		0.65		0.65		0.43	± 0.16
V_f	52.5		111.9		94.5		70.0		79.2	± 15.2
V_f/kg	1.05		1.40		1.18		1.03		1.10	± 0.12
k_{el}	123.1		219.3		359.3		212.6		259	± 69
k_{el}/kg	2.46		2.74		4.33		3.13		3.57	± 0.83
r_{12}	0.004		0.013		0.009		0.007		0.032	± 0.034
r_{21}	0.006		0.006		0.012		0.012		0.014	± 0.005
k_{12}	130		458		506		300		694	± 384

rapid initial drop in plasma concentration after termination of the infusion, due to the distribution of the drug into the peripheral compartment. Only a limited number of plasma samples can be obtained during this short period of time. Consequently this is the main explanation for the relatively large errors in the parameters describing this phase. Usually, the errors in A_2 and τ_2 were much smaller and this substantiated the evidence for the single first-order kinetics of the elimination phase. The plasma half-life of hexobarbital in man is seen to vary from 160 to 441 min, with a mean value of 261 min. The main factors governing the rate of elimination of the drug in the body are the metabolic clearance (k_{el}) and the apparent volume of distribution (V_f). This volume (per kg body-weight) is relatively constant in the subjects, whereas there is a substantial variability in the metabolic clearance constant (Table I). It can be concluded therefore that the observed differences in half-life are mainly due to individual differences in the hexobarbital metabolizing ability of the liver. Siegert et al. (1964) found also a substantial individual variability of $t_{1/2}$ in dogs

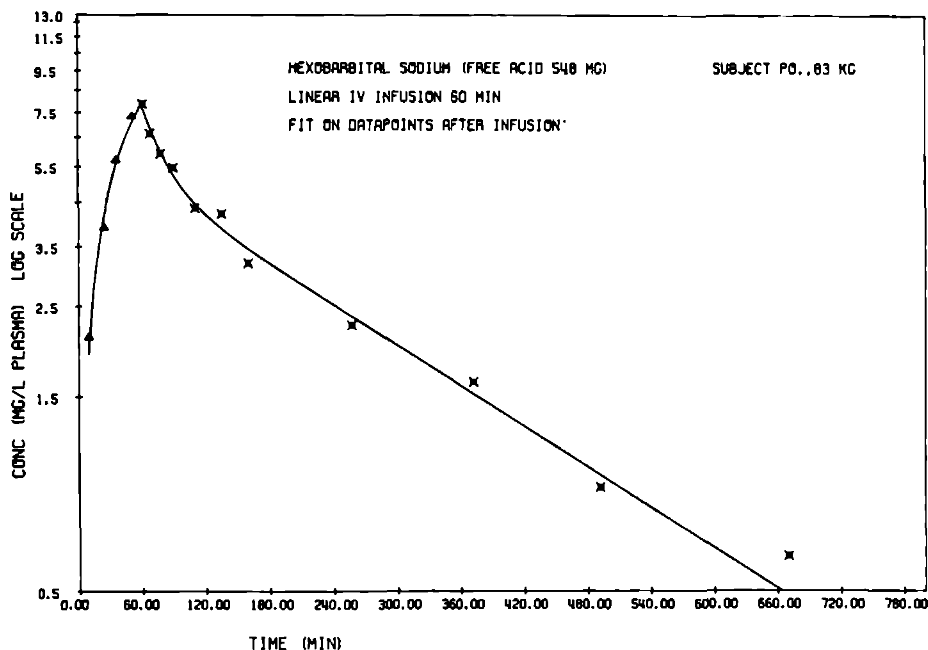

FIG. 3. Hexobarbital plasma concentration curve on semi-logarithmic scale during and after a 60 min zero-order i.v. infusion of hexobarbital sodium into a healthy volunteer. Fitting and plotting occurred in the same way as described for Fig. 1. It can be noted that with a 60 min infusion the plasma concentrations during infusion remain much lower than in the case of a 30 min infusion.

for hexobarbital, which they attributed to fluctuations in the amount of drug metabolizing microsomal protein.

The calculated parameters V_1 (the volume of the central compartment) and r_{12} (the distribution rate constant from the central to the peripheral compartment) vary considerably due to the fact that their calculation is strongly dependent upon A_1 and τ_1. It is clear, however, that the volume of the central compartment is a factor two to three fold smaller than the overall apparent volume of distribution V_f. This indicates extensive tissue distribution of the drug, which may be explained by its relatively high lipophility. The smaller distribution rate contstant from the tissues to the plasma compartment (r_{21}), when compared with r_{12}, is a consequence of the apparent large volume of the tissue compartment.

All of the volunteers displayed typical signs of central nervous system depression during infusion (drowsiness, incoherent speech and sometimes sleep). These effects, which varied considerably in intensity from one person to the other, became more pronounced as the infusion proceeded. This was evident especially in the case of a 30 min infusion. Obviously, when infusion proceeds more rapidly the plasma concentration and also the brain concentration for this lipophilic drug rise more rapidly. The peak plasma concentrations were approximately 1.5 fold higher with a 30 min infusion than in the case of a 60 min infusion (compare Fig. 1 to Fig. 3). After infusion recovery occurred relatively rapid and all the subjects were able to go out for lunch within 4 hours after drug administration. The plasma levels attained at this time were usually in the range of 2 - 4 mg/l.

TABLE II. Cumulative renal excretion (24 h) of hexobarbital and 3-keto-hexobarbital after i.v. administration of hexobarbital sodium

Subject	Dose administered (mg free acid)	Hexobarbital		3-keto-hexobarbital	
		mg	% of dose	mg	% of dose
W.G.	585.6	1.84	0.31	270.8	43.7
B.B.	534.4	1.37	0.26	221.3	39.1
D.A.	505.1	2.43	0.48	335.1	62.6
S.G.	366.0	1.51	0.41	148.4	38.3
B.E.	505.1	1.31	0.26	156.2	29.2
Mean percentages			0.34		42.6

The results of the 24 hours' urinary excretion of unchanged drug and 3-keto-hexobarbital for five of the participants are summarized in Table II. Hexobarbital is excreted unchanged only to a very small extent; it accounts for less than 0.5% of the administered dose. This is explained by its lipophilic properties and the relatively high pK_a-value, which make reabsorption from the ultrafiltrate in the kidney self-evident. A metabolite which has been detected in the urine of several species, after hexobarbital administration, is 3-keto-hexobarbital (Bush and Weller, 1972). Tsukamoto et al. (1957) and Frey et al. (1959) identified this compound in the urine of man, but no quantitative data on the renal excretion are available. The present findings suggest that 3-keto-hexobarbital is an important metabolite in quantitative terms, since its excretion accounts for 29 - 63% of the dose administered in 24 h. Information on the renal excretion rate of this metabolite will be given in Chapter 2 of this Section.

DISCUSSION

The first-order elimination of racemic hexobarbital is surprising, since in a previous investigation (Breimer and van Rossum, 1973; Chapter 2, Section III), it was found that the optical isomers of hexobarbital are eliminated with different rates in man. The (+)-isomer showed an average half-life of 4.6 h and the (—)-isomer of 1.4 h. As a consequence of this 3-fold difference in elimination rate, the elimination phase of the racemic mixture should not give a straight line from theory. Two different time constants are underlying this phase for the racemate. However, it can be argued that if a drug with a half-life of 1.4 h is infused in a time period of 30 min or 1 h, then a great deal has already been eliminated during infusion and during the distribution phase. When the pure elimination phase is reached, the contribution of the (—)-isomer may be almost negligible. This is in agreement with the fact that the estimated half-life of the racemate is almost equal to the half-life of the (+)-isomer (4.4 h versus 4.6 h). In fact, the half-life of the (+)-isomer is being determined by the present experimental procedure. Only when the racemic mixture of hexobarbital is administered by rapid i.v. infusion it may be possible to distinguish between the half-lives of the two enantiomers (Chapter 2, Section III). It should be realized that in the present experiments the infusion phase and distribution phase are in fact complex functions of the rapid elimination of (—)-hexobarbital, the slower elimination of (+)-hexobarbital and the distribution of the two compounds into peripheral tissues.

The present pharmacokinetic data may provide information on the role of adipose tissue in hexobarbital elimination. Brodie et al. (1950, 1952) showed

that thiopental is taken up by adipose tissue to a considerable extent. Price et al. (1960) found that the uptake was a slow process, the equilibrium between plasma and adipose tissue was reached after 4 -5 h. The previously reported data were reconsidered by Bischoff and Dedrick (1968) and it follows from their calculations, that thiopental kinetics should be consistent with the three-compartment open model. After 4 - 5 h a distinct slower phase may predominate the elimination process, which is due primarily to redistribution of the drug from adipose tissue. The affinity of hexobarbital to fat is less than thiopental (Brodie, 1952), whereas it has a relatively high susceptibility for metabolic degradation (see clearance values). It is the combination of these two properties which is responsible for the fact that adipose tissue plays a minor role in the elimination of hexobarbital. At later times following drug administration there were no indications for a slower elimination phase than that corresponding to an average half-life of about 4 h. It seems likely that lean tissues (e.g. muscle, connective tissue, bone, lung, skin), are primarily responsible for the rapid depletion of hexobarbital from the central nervous system after i.v. administration, similar to thiopental (Price et al., 1960). This is an explanation for the rapid regain of consciousness after rapid i.v. application for anaesthesia. Furthermore, it can be concluded from the present experiments that metabolism is also a main factor in the termination of CNS depressant action. The half-life of hexobarbital is such that it may be suited for hypnotic drug therapy. After a 9 hours' night's rest approximately 75% of the administered dose has been inactivated. Thus, the risk of residual effects is small, provided that a normal dose has been administered.

C. ORAL AND RECTAL ADMINISTRATION

METHODS

Composition and preparation of the dosage forms

Seven dosage forms, containing hexobarbital and hexobarbital sodium were used in the present investigations. The compositions are outlined in Table III. The EVIPAN-tablets were obtained commercially and the other dosage forms were prepared at the Dutch Pharmacist's Laboratory, The Hague. The hexobarbital or hexobarbital sodium content was determined by the bromometric method of the National Formulary XII. The tablet content was determined acidimetrically.

TABLE III. Composition of the dosage forms containing hexobarbital or hexobarbital sodium.

	Capsule				Tablet	Suppository	
	I	III	V	VI	VIII[9]	IV	VII
Hexobarbital[1]	300 mg	300 mg	–	–	250 mg	600 mg	–
Hexobarbital sodium[2]	–	–	300 mg	300 mg	–	600 mg	–
Colloidal silica[3]	1.7 mg	1.7 mg	3 mg	3 mg	–	–	6 mg
Methylcellulose 400cp[4]	–	1.4 mg	–	–	–	–	–
Microcrystalline cellulose[5]	60 mg	60 mg	–	–	–	–	–
Dried potato starch[6]	–	–	60 mg	–	–	–	–
Heavy magnesium oxide	–	–	–	60 mg	–	–	–
Adeps solidus[7]	–	–	–	–	–	1.4 g	1.4 g
Content (%)[8]	98	100	96	97	99	109	96

[1]) Onderlinge Pharmaceutische Groothandel, Utrecht; [2]) Siegfried S.A., Switzerland; [3]) AEROSIL 200; [4]) METHOCEL 400 MC Dow Chemical; [5]) AVICEL pH 101; [6]) water content 8 per cent; [7]) WITEPSOL H15, Chemische Werke Witte; [8]) actual hexobarbital content in percent of stated content; [9]) EVIPAN, Bayer A.G.

Hexobarbital acid was passed through a 150 μm mesh sieve before mixing with the additives in preparation I, III and IV. Hexobarbital acid is only slightly soluble in water (1 g in 3000 ml according to Martindale, 1972). It was tried in capsule III to increase hydrophility of this compound by moistening with a 3% solution of methylcellulose in water. The mixture was passed through a 300 μm mesh sieve, dried at 40° C and mixed with the other constituents (Cox and Smulders, 1969).

Hexobarbital sodium is very hygroscopic and readily soluble in water. Therefore additives were used which are effective water absorbents (magnesium oxide in capsule VI and dried potato starch in capsule V). Mixing was accomplished with a ball mill in order to prevent absorption of air moisture. The capsules (no. 0) were filled with a Feton fill-and-close hand apparatus.

Suppositories IV were prepared by mixing the drug substance with the melted fatty base (Witepsol H 15). The mixture was poured into 2 ml moulds. Suppositories VII were made in the same way using a mixture of hexobarbital sodium and colloidal silica prepared in a ball mill. Witepsol H 15 is a mixture of mono-, di- and triglycerides with a melting range of 33.5 - 35.5° C, congealing range of 32.5 - 34.5° C, a specific gravity at 20° C of 0.950 - 0.980, an iodine number less than 7, a saponification value of 230 - 240 and a hydroxyl value less than 15.

All preparations were stored in well-closed containers and protected from light. Hexobarbital sodium capsules were stored in well closed bottles containing anhydrous silicagel in the snap cap.

In vivo experiments

Twenty-eight healthy male volunteers, ranging in age from 18 - 26 years and in body-weight from 58 - 86 kg, participated in the study. They had received no regular medication during the 4 weeks preceding the initiation of the experiments. After an overnight fast, at 9 a.m., the subjects were given 600 mg hexobarbital or 600 mg hexobarbital sodium, together with 150 ml water. Two of the capsules, $2^2/_5$ of the tablets and one suppository were required to achieve a dose of 600 mg (Table III). Initially, the volunteers were asked to remain in an upright position for about 15 min and then to lie down for at least 3 or 4 h. This procedure was undertaken in order to simulate as closely as possible the situation of taking a hypnotic drug and going to bed. No food, fluid or tobacco was allowed for at least 3 h after drug administration. Blood samples were taken from a forearm vein at frequent times during the first 4 h. These differed somewhat for the various dosage forms but they can be deduced from

the plasma concentration curves given in Fig. 4 to 7. For several volunteers blood samples were taken also at later times, in order to determine the plasma half-life for hexobarbital. Hexobarbital plasma concentrations were measured by gas chromatography, as described in Chapter 2, Section II.

Some volunteers participated several times during the present study; they received either the same dosage form or an alternative one. Intervals between any two trials were at least one week. Three of the volunteers who had received a suppository containing 600 mg hexobarbital sodium, also received a suppository containing 100 mg hexobarbital sodium in order to study the influence of the dose on the absorption rate and the relative bioavailability after rectal administration.

Participation in a study of repetitive hexobarbital administration is recorded for two volunteers. On the first day of the experiment the half-life of the drug was determined by administering 300 mg hexobarbital orally and taking blood samples at regular times. From the first night the volunteers took 300 mg hexobarbital sodium (capsule VI) with 150 ml water, each night. The next morning, nine hours after drug intake, a blood sample was taken usually and the hexobarbital plasma concentration was determined. After 9 days hexobarbital was given once again in the morning in order to determine the half-life of the drug after repetitive administration. In Fig. 10 the time schedule of drug administration and blood sampling is indicated.

RESULTS

The plasma concentrations in the present experiments were in many cases only followed up to 3.5 or 4 h after drug administration, since the primary aim of this investigation was to ascertain the differences in hexobarbital absorption rate from the various dosage forms. In Fig. 4 to 7 all the individual plasma concentration curves are given for the seven preparations. Three important observations can be made: a. there exists a substantial intersubject variation in absorption profile for each dosage form; b. hexobarbital reaches the general circulation more rapidly when it is administered as the sodium salt; c. plasma concentrations during the first 4 h remain much lower after rectal administration when compared with oral administration. Also, for each preparation the average absorption profile was estimated (Fig. 8). It appears that the mean curves of capsules V and VI (containing hexobarbital sodium) and also of capsule III (containing hexobarbital free acid) show two maxima, whereas almost none of the individual curves shows such a profile. This is explained by the fact that in some volunteers relatively rapid absorption occurs,

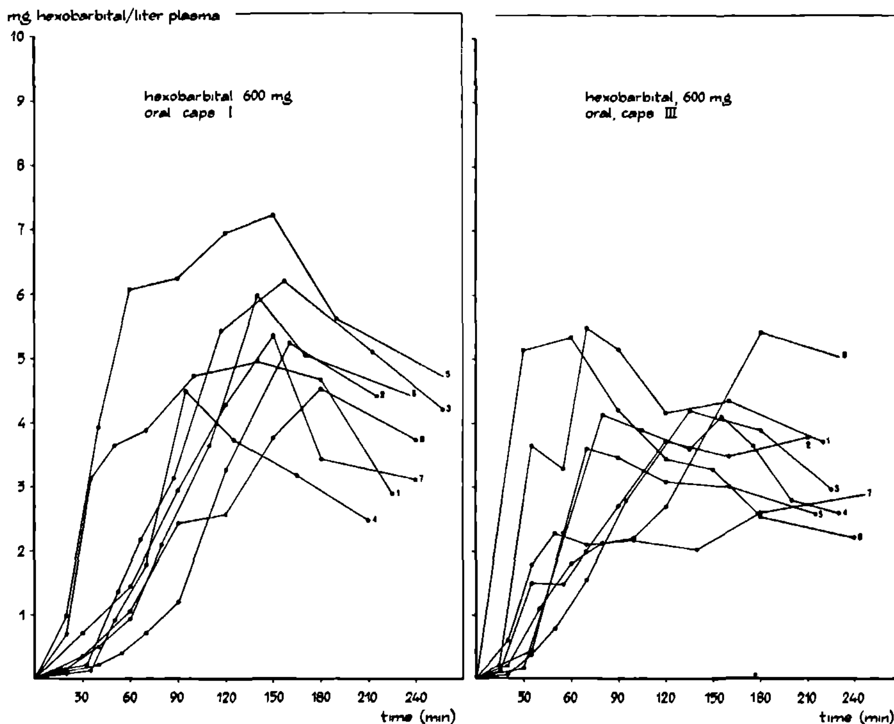

FIG. 4. Hexobarbital plasma concentration curves after oral administration of capsule I (*left*) and capsule III (*right*).

while in others, receiving the same dosage form, this was substantially slower. In this way the „unnatural" average curves are obtained. It may be concluded that average values alone do not yield appropriate information in a study such as this.

As already mentioned, in most experiments the plasma concentrations were not followed long enough in order to permit the individual half-life to be determined. In consequence it was impossible to estimate the half-life of absorption and the lag time for each curve. Therefore two other parameters, characterizing the absorption process are given in Table IV, which are the peak concentration (C_{max}) and the peak concentration time (t_{max}). The suppositories have not been included in this Table, since absorption had not been completed after 4 h (Fig. 7). With respect to t_{max} and C_{max}, the oral preparations with hexobarbital free acid were significantly different from capsule VI, but not from

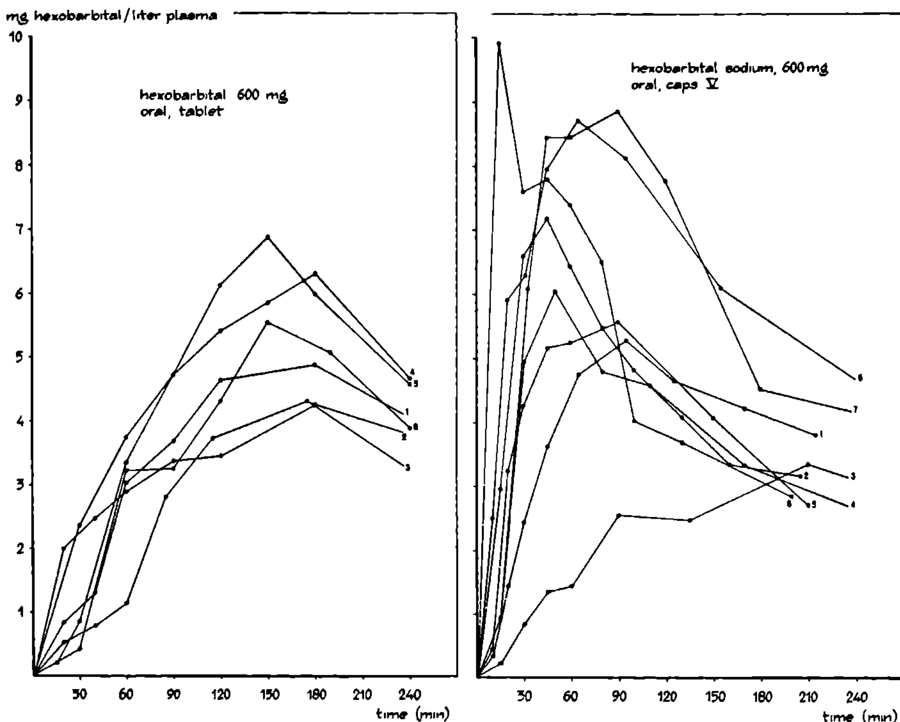

FIG. 5. Hexobarbital plasma concentration curves after oral administration of tablet VIII (*left*) and capsule V (*right*).

capsule V. Differences among the free acid containing dosage forms were not statistically significant ($P > 0.05$) except for C_{max} between capsule I and III. Also, for preparations V and VI no significant difference became apparent.

In many cases where the hexobarbital sodium capsules (V or VI) were administered, the subjects felt a strong tendency to sleep, within 15 - 30 min after drug intake. Feelings of euphoria were reported often. All evidence for CNS depression was registered and afterwards it could be deduced from the individual plasma concentration curves that they occurred around the time of the peak concentration. Subjective recovery was apparently complete within 4 - 5 h after drug administration. With the free acid containing dosage forms the CNS depressant effects were less pronounced and feelings of sleepiness became apparent mostly after 1 - 2 h. None of the volunteers receiving a suppository experienced CNS depressant feelings. In a separate study, at the

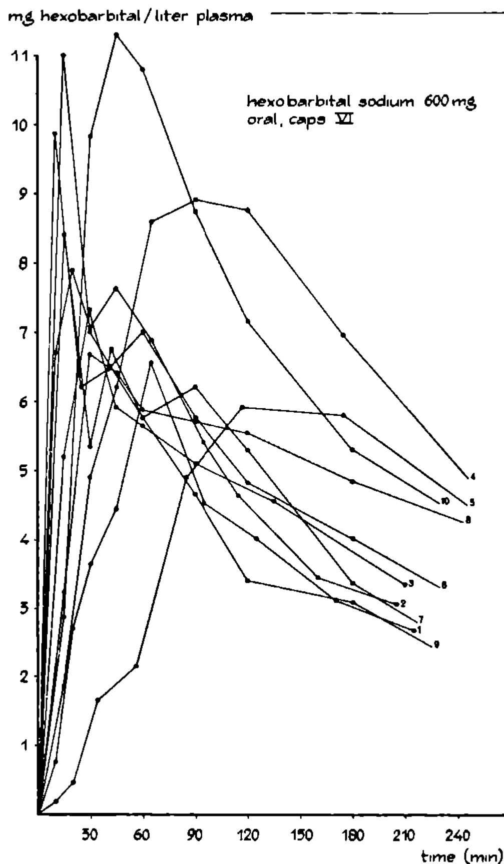

FIG. 6. Hexobarbital plasma concentration curves after oral administration of capsule VI.

Institute for Perception TNO at Soesterberg (Trumbo and Gaillard, 1974), it was found that volunteers receiving hexobarbital sodium in a suppository (dosage form VII) showed reduced effects of immediate arousal to auditory signals, compared to a placebo session. This objective assessment of an impaired performance with this suppository may indicate that an average plasma concentration of 2 mg/l is minimally effective for hexobarbital, with respect to CNS depression. It seems likely that somewhat higher concentrations are required for the virtual experience of sedation or sleepiness.

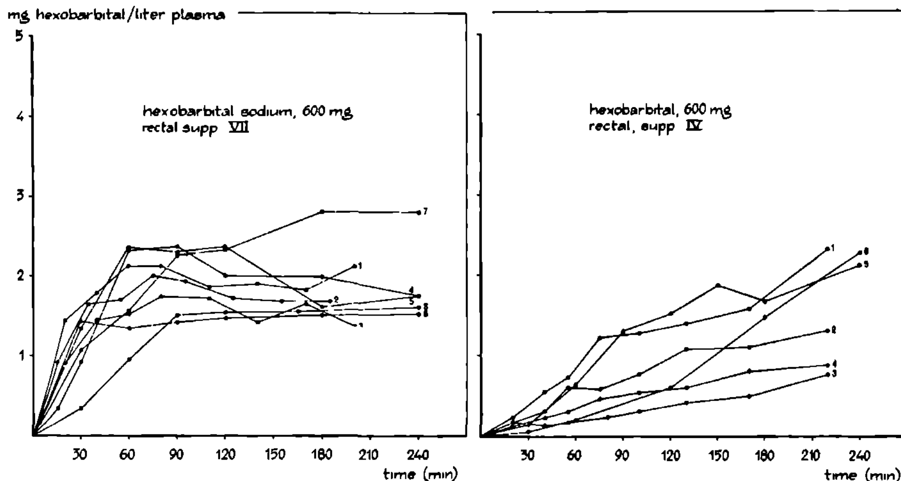

FIG. 7. Hexobarbital plasma concentration curves after rectal administration of suppository VII (*left*) and suppository IV (*right*).

With respect to the bioavailability of hexobarbital from the various dosage forms the following equation applies, assuming linear kinetics:

$$F \times D = k_{el} \cdot \int_0^\infty C_p dt = k_{el} \cdot AUC$$

where F = bioavailability ($0 \leqslant F \leqslant 1$), D = dose administered, C_p = plasma concentration, k_{el} = total body clearance constant, and AUC = area under the plasma concentration curve from $t = 0$ to $t = \infty$. The clearance constant can only be estimated accurately after i.v. administration ($F = 1$). However, it may be assumed that the average clearance value was about the same for each group of volunteers receiving one of the dosage forms. An indication that this assumption is valid was found in the fact that the hexobarbital elimination half-lives, determined in some of these subjects, showed in general no significant differences between the various groups. The average relative bioavailability of hexobarbital from each dosage form can be calculated, taking capsule VI as the reference:

$$F_{rel} = \frac{AUC_x}{AUC_{VI}}$$

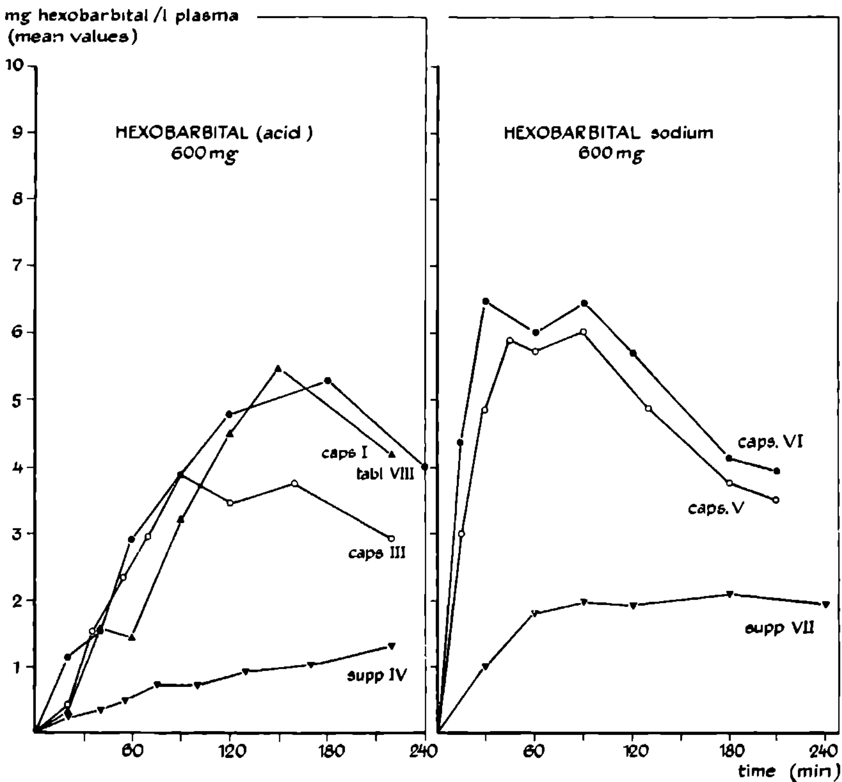

FIG. 8. Mean hexobarbital plasma concentration curves following administration of the seven hexobarbital or hexobarbital sodium containing dosage forms.

The plasma concentrations were measured up to 4 h after administration and consequently the AUC could be determined only up to this time. Therefore a correction had to be made for the remaining area. If at the time of the last plasma concentration, measured at time t = t′, absorption has been completed, the infinite area is given by the following equation (Dost, 1968):

$$\int_0^{\infty} C_p dt = \int_0^{t'} C_p dt + \int_{t'}^{\infty} C_p dt = AUC\Big/_0^{t'} + C_p^{t'} \cdot \tau_{el}$$

where τ_{el} is the elimination time constant and $C_p^{t'}$ is the last measured plasma concentration. The AUC's from t = 0 to t = t′ of the mean curves in the present study were determined by weighing the corresponding areas under the

TABLE IV. Absorption characteristics of hexobarbital and hexobarbital sodium after oral administration[1])

Dosage form	Number of experiments	Dose (mg free acid)	Mean t_{max} ± S.D.[2]) (min)	Mean C_{max} ± S.D.[2]) (mg/l)
Capsule I	8	600	147 ± 24	5.48 ± 0.93
Capsule III	8	600	129 ± 63	4.34 ± 0.98
Tablet VIII	6	600	169 ± 15	5.36 ± 1.07
Capsule V	8	549	83 ± 58	6.86 ± 2.20
Capsule VI	10	549	50 ± 36	8.39 ± 1.87

1) t_{max} refers to the time after which the maximum plasma concentration (C_{max}) is reached.

2) For t_{max} and C_{max} statistically significant differences at $P < 0.01$ (Student's t-test) were found between dosage forms I and VI, III and VI, VIII and VI. Also a significant difference at $P < 0.01$ was found for t_{max} between dosage form VIII and V; at $P < 0.05$ for t_{max} between I and V; at $P < 0.05$ for C_{max} between I and III.

TABLE V. Relative bioavailability of hexobarbital from the different dosage forms after oral administration

Dosage form	Dose (mg free acid)	t'[1]) (min)	AUC 0-t' (mg.min/l)	conc. at t' (mg/l)	AUC 0-∞[2]) (mg.min/l)	F_{rel}[3]) (%)
Capsule I	600	220	719	4.2	2298	89
Capsule III	600	220	613	3.0	1741	63
Tablet VIII	600	240	884	4.0	2388	86
Capsule V	549	210	931	3.5	2247	89
Capsule VI	549	210	1069	3.9	2535	100

1) Time at which the last hexobarbital plasma concentration was measured.

2) These values were obtained by multiplying the concentration at t' by the average elimination time constant (376 min) and adding the result to AUC 0-t'.

3) The bioavailability was calculated relative to capsule VI and corrected for the differences in dose, assuming a linear relationship between dose and AUC; the dose was not corrected for the assayed content of the dosage forms.

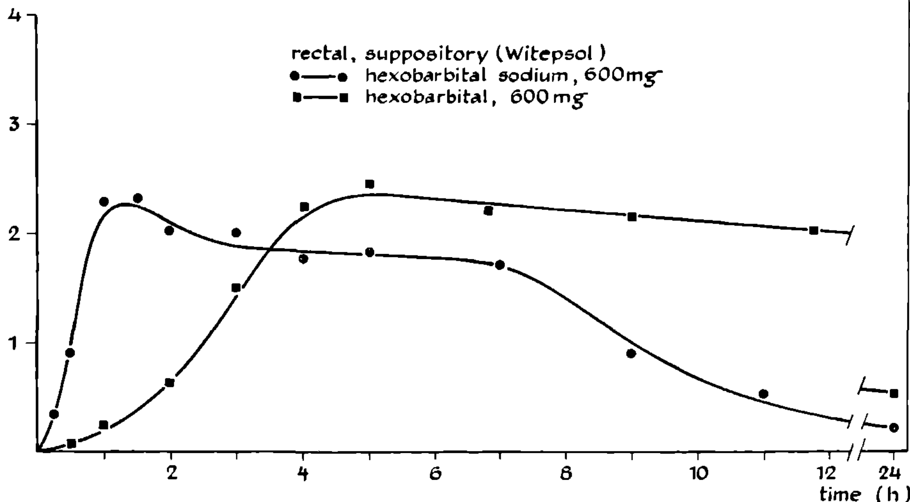

FIG. 9. Hexobarbital plasma concentration curves after rectal administration of suppository IV and VII to the same volunteer. Note that in both cases a relatively constant plasma level results during a few hours.

plasma concentration curves, whereas the remaining areas from t = t′ to t = ∞ were calculated by multiplying the concentrations at t = t′ by τ_{el}. For the latter value the mean τ_{el} after i.v. hexobarbital administration was taken (376 min). In Table V the results are given for the oral preparations. The suppositories were omitted because absorption was not complete when the plasma concentration measurement was stopped. It follows that only the dosage form III as a hexobarbital delivery system is inferior when compared to the other dosage forms.

As mentioned before, absorption from the suppositories was not complete within 4 h after administration (Fig. 7). Therefore some experiments were undertaken to follow hexobarbital plasma concentrations for a longer time period after the administration of these formulations. In Fig. 9 an example is given of the curves obtained. An interesting fact is that in both cases the plasma level remained relatively constant for a few hours. A bioavailability relative to capsule VI of 40 - 60% was estimated for both types of suppositories (determined in three volunteers), which is substantially lower than after oral hexobarbital administration. Since the possibility existed of the amount of drug influencing bioavailability, further experiments were carried out with three

TABLE VI. Relative bioavailability of hexobarbital from suppositories, containing 600 or 100 mg of hexobarbital sodium, after administration to the same volunteers.

Subject	Dose (mg)	t' [1] (h)	AUC 0-t' (mg.min/l)	conc. at t' (mg/l)	τ_{el} [2] (min)	AUC 0-∞ (mg.min/l)	F_{rel} [3] (%)
S.H.	600	11	972	0.58	270	1128	72
	109 [4]	9	240	0.18	270	288	100
E.G.	600	12	900	0.69	342	1134	91
	109	9	160	0.22	342	228	100
W.E.	600	12	1158	0.82	354	1446	83
	109	9	300	0.31	354	312	100

[1] Time at which the last hexobarbital plasma concentration was measured.

[2] The elimination time constant was obtained from the descending part of the plasma concentration curve after administration of the suppository with the higher dose. It was assumed that the time constant was the same at the second occasion.

[3] The bioavailability was calculated relative to the suppository containing 109 mg hexobarbital sodium and corrected for the difference in dose, assuming a linear relationship between dose and AUC.

[4] The assayed content appeared to be 109 mg hexobarbital sodium.

volunteers who had received a suppository containing 600 mg hexobarbital sodium previously and who also received a suppository with 100 mg of the same substance. Plasma concentrations were measured up to 12 h and the bioavailability relative to the suppository with 100 mg, was calculated for each individual, as indicated in Table VI. It follows that for the higher dose, absorption efficiency (bioavailability) diminishes which indicates that the amount of hexobarbital in a suppository influences bioavailability.

During repetitive hexobarbital administration (300 mg each night) accumulation of the drug in the body did not occur (Fig. 10), which is in agreement with its short elimination half-life. Plasma concentrations 9 h after drug intake were always in the same range and had decreased below 0.5 mg/l. The half-life after 10 days had not changed, which indicated that no induction of drug metabolizing enzymes had occurred. Barbiturates are considered to be potent inducers of the microsomal enzymes (Remmer, 1972), also with respect to the acceleration of their own metabolism. Hexobarbital probably does not remain, however, in sufficiently high concentrations in the body to cause such an induction. Further investigations are required to see whether higher doses

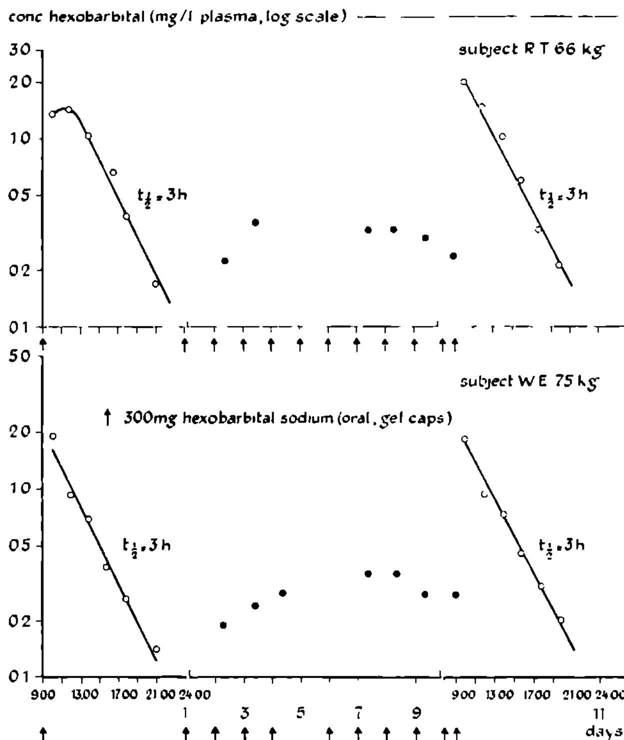

FIG. 10. Plasma concentrations of hexobarbital during and after repetitive hexobarbital administration to two volunteers. During the first and the last day of the experiment the half-life of the drug was determined. From the first night a capsule (preparation VI), containing 300 mg hexobarbital sodium, was taken each night and usually the next morning (9 h later) the plasma concentration was measured. Note that these concentrations were always in the same range. Also note that the plasma half-life after repetitive hexobarbital administration was the same as prior to the experiments.

and more frequent administration of hexobarbital will accelerate drug metabolism.

DISCUSSION

Hexobarbital versus hexobarbital sodium

One of the most evident results of the present investigation is the difference in absorption rate between hexobarbital and hexobarbital sodium. The rate of

absorption for many drugs is limited by the rate of dissolution in the gastrointestinal fluid, because a drug has to be dissolved before it can pass through a biological membrane (Garrett, 1971; Benet, 1973). Hexobarbital free acid is very slightly soluble in aqueous fluids, whereas its sodium salt is readily soluble. This explains the more rapid absorption when the salt form is administered. In vitro dissolution experiments (Levy beaker method; 500 ml 0.08 N hydrochloric acid; 80 r.p.m.; 37° C) revealed a relatively rapid dissolution for the hexobarbital sodium containing dosage forms and slower dissolution for the free acid (Cox et al., 1974). Higuchi et al. (1958) discussed that salts generally will show higher dissolution rates than the corresponding non-ionic drug at any pH, even though the final equilibrium solubility of the drug and its salt would be the same. This is explained by the fact that if precipitation occurs, generally a suspension of fine particles results, which has the proper characteristics for rapid redissolution. This phenomenon apparently applies in the case of barbiturates, since their sodium salts will be precipitated in the acid medium of the stomach, but nevertheless a higher absorption rate is observed than with the corresponding free acids. Sjögren et al. (1965) demonstrated this for several barbiturates including hexobarbital. In Chapter 5 of this Section similar findings are reported for heptabarbital and heptabarbital sodium. Also, the very rapid absorption of hexobarbital sodium in aqueous solution is in agreement with the above hypothesis (Bush et al., 1966). The present experiments indicate that the absorption of hexobarbital is dissolution rate limited, when the drug is administered orally as the free acid.

Careful analysis of the individual plasma concentration curves revealed that in cases where very rapid absorption had occurred (capsule VI or V), the post absorption phase consisted of a rapid drop in plasma concentration followed by a slower decline. This behaviour suggests that now absorption had occurred more rapidly than the distribution of the drug into tissues. The shape of the curve is consistent with two-compartment kinetics, similar to hexobarbital kinetics after i.v. infusion. After oral administration of pentobarbital sodium, Smith et al. (1973) also found a bi-exponential plasma concentration decay after absorption. However, in this case the „distribution phase" lasted longer than with hexobarbital, possibly due to the less lipophilic character of pentobarbital.

Influence of dosage form composition

Part of the present investigation was devoted to the influence of some additives on the absorption rate and bioavailability of hexobarbital. As was pointed

out in Chapter 2 of Section I, formulation factors may greatly influence the amount and the rate by which a drug substance reaches the general circulation. The results with hexobarbital suggest that the main differences between the dosage forms used, are due to differences in aqueous solubility between hexobarbital sodium and the free acid. The differences between capsule V and VI, both containing the sodium salt, were not statistically significant. Yet, capsule VI is preferred since the differences for t_{max} between the free acid containing dosage forms and capsule VI were statistically significant, whereas they were not for capsule V. Besides, dissolution data (Cox et al., 1974) predicted a more rapid absorption for preparation VI. It appears likely that magnesium oxide (capsule VI), which has basic properties, partly neutralizes gastric juice when entering the stomach, thereby creating an environment which favours the dissolution of hexobarbital sodium or redissolution of precipitated free acid. The potato starch (capsule V) on the other hand has not such an influence and certainly does not enhance redissolution consistently. In conclusion, capsule VI is the hexobarbital sodium containing dosage form of choice.

With respect to hexobarbital free acid, an attempt to increase the dissolution rate of this hydrophobic compound was made by granulation with a 3% methylcellulose solution (capsule III). The initial in vitro results showed that the dissolution rate of capsule III was substantially higher than that of capsule I, indicating effective hydrophilization by methylcellulose granulation. This resulted in the prediction that hexobarbital capsule III should give better in vivo results than capsule I, yet the opposite became apparent. The plasma concentration curves obtained after administration of capsule III varied widely, some of which indicated relatively rapid absorption and others quite slow (Fig. 4). Besides, the average relative bioavailability of this preparation was substantially lower when compared with the other dosage forms. The present findings illustrate the problem of predicting even the rank order relationship of plasma levels from the in vitro dissolution data. It is possible that some of these discrepancies were due to the fact that the dissolution study was run too close to saturation concentration (Riegelman, personal communication). However, the reason for the unexpected in vivo results is difficult to understand. Previously granulation with gelatine was shown to increase the absorption of secobarbital (Sjögren, 1971), and in fact the same principle was applied in the present study. It is possible that the granulation procedure and the amount of methylcellulose are critical factors.

The composition of the tablets is unknown, but it can be observed that its absorption behaviour is quite regular (Fig. 5). There are no essential differences between capsule I and the tablets, so there is no question of any preference between them.

A variety of factors, physiological and pharmaceutical, may influence the rectal absorption of drugs (Wagner, 1971; Bevernage and Polderman, 1973). The physico-chemical properties of the drug substance and of the vehicle in which the drug is incorporated are very important. Significant variations in absorption rate and bioavailability have been encountered by changing for example the suppository base containing the same amount of drug (Kerckhoffs and Huizinga, 1967). There are two main types of bases; those which are lipophilic in nature (cacao butter, synthetic mixtures of triglycerides, etc.) and those which are hydrophilic (e.g. polyethylene glycols). For the present experiments a lipophilic base was chosen, since Higuchi and Lach (1954) have reported that barbiturates may complex with polyethylene glycols, which results in reduced dissolution and absorption rates (Singh et al., 1966). However, it should be mentioned that the investigations with vinylbital, after rectal administration (Chapter 4, Section III), revealed that absorption from a polyethylene glycol base will not necessarily result in a greatly reduced absorption rate or bioavailability.

It was previously shown in rabbits that the sodium salts of barbiturates are more rapidly absorbed from lipophilic bases than the corresponding free acids (Samelius and Aström, 1958; Neuwald et al., 1962; Fincher et al., 1966). The present results, for hexobarbital in man, are consistent with these earlier findings and indicate that aqueous solubility plays an important role also in rectal absorption. Hexobarbital free acid absorption is very slow, since its partitioning from the lipophilic Witepsol base into the aqueous fluid of the rectum is very unfavourable. The interesting behaviour of both preparations, which exhibit a constant plasma level for a few hours (Fig. 9), indicates that the amount of drug absorbed per unit of time during this period, is equivalent to the amount being eliminated per unit of time. This is only possible if absorption during this period is an apparent zero-order process. For the free acid it is assumed that dissolution is in principle a first-order process. However, a severely limiting factor may be the amount of fluid in the rectum, which is only 1 - 3 ml (Bevernage and Polderman, 1973). The saturation concentration is readily reached and the amount dissolved becomes equivalent to the amount absorbed per unit of time. For the sodium salt there are probably no dissolution problems, as the pH in the rectum is about 7 and will be substantially raised by the presence of the salt. Since only the unionized form of a drug can pass through biological membranes, a high pH is unfavourable for the absorption of barbiturates. This factor, together with the limited absorption surface, may

be the underlying cause for the apparent zero-order absorption of the sodium salt. The dose may also be important in this respect since the plasma concentration curves obtained after 100 mg hexobarbital sodium revealed a normal behaviour, with a distinct absorption and elimination phase. On only one occasion was a constant plasma level evident for some time. A high dose is also unfavourable with respect to bioavailability (Table VI).

In conclusion, the present findings emphasize that the rectal route of administration cannot be looked upon as a routine alternate route of administration of drugs in general. The situation is more complicated than after oral administration and therefore appropriate in vivo data have to be provided when the rectal route is recommended (Wagner, 1971). Once the absorption profile of a certain dosage form has been established, it can be judged for which therapeutic purposes, the formulation may be used. In the case of hexobarbital, for instance, the suppositories could be applied in sedative drug therapy, since the plasma levels remain constant for quite a long time. They are on the other hand not suitable in the treatment of insomnia.

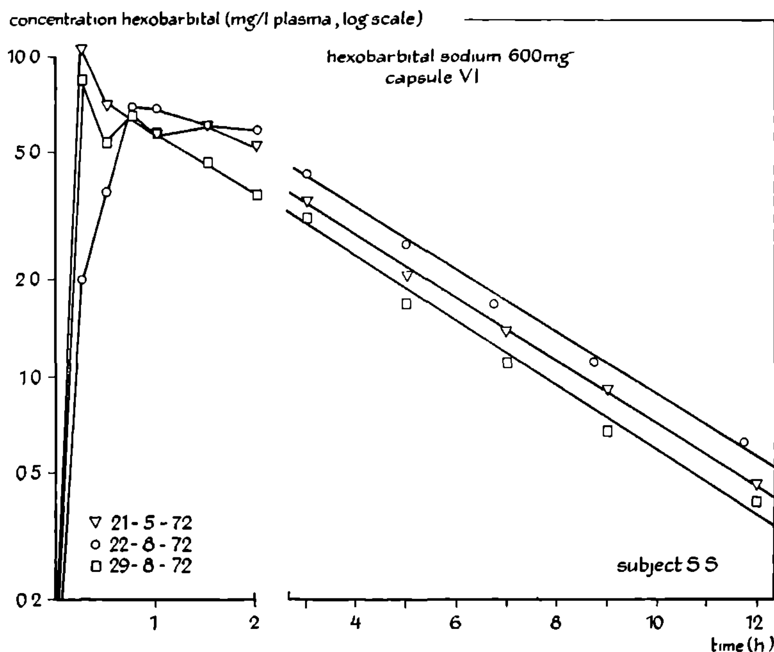

FIG. 11. Hexobarbital plasma concentration curves following oral administration of capsule VI at three different occasions to the same volunteer. Note the variation in absorption profile and the constancy of the hexobarbital half-life.

It may be observed from the individual plasma concentration curves that there was a substantial intersubject variation with respect to hexobarbital absorption from each dosage form. These differences could not be related to differences in body-weight and may be explained only in part by differences in elimination half-lives. It cannot be excluded that on some occasions fasting regulations were not obeyd strictly. Food will undoubtedly retard the rate of absorption, as was found in preliminary experiments with hexobarbital sodium. The question arose of how constant the absorption profile of the same dosage form would be, when administered twice to the same volunteer under identical circumstances. Some additional experiments were undertaken and the dosage forms were administered with an interim of one week. The results revealed substantial intrasubject variation, especially with the hexobarbital sodium containing dosage forms. An example is given in Fig. 11. With the free acid differences in plasma concentrations between the two occasions were considerable during the absorption phase. Changes in bioavailability were not very great (10 - 15%). It is concluded that considerable intrasubject variation exists with respect to hexobarbital or hexobarbital sodium absorption. Obviously with this information available, the even greater intersubject variation may be understood. It is interesting to note that the elimination half-life of hexobarbital in

TABLE VII. Comparison of hexobarbital half-lives in the same volunteer determined at different times[1])

Subject									
M.S.		S.S.		F.P.		E.W.		J.K.	
$t_{1/2}$[2])	Δt[3])	$t_{1/2}$	Δt	$t_{1/2}$	Δt	$t_{1/2}$	Δt	$t_{1/2}$	Δt
4.7	0	3.0	0	3.2	0	3.6	0	4.7	0
5.4	12	3.2	14	3.3	20	4.3	20	4.1	22
4.1	31	3.0	29	3.8	21	4.1	21	4.1	23
4.5	32	3.3	30	3.4	26				
4.3	37								

[1]) The half-lives were determined after oral or i.v. administration of hexobarbital or its sodium salt.

[2]) Plasma half-life in h.

[3]) Interim in weeks of the separate half-life determinations, reckoned from $\Delta t = 0$.

the different experiments was found to be quite constant for the same volunteer (Fig. 11). In a recent article Wagner (1973) pointed out that there may be appreciable intrasubject variation in elimination half-life for drugs which are completely metabolized by man. When hexobarbital was given with an interim of one week to the same volunteer the difference in half-life did not exceed 10% in general. This variation must be considered as falling within the experimental error and the half-life should be regarded therefore as unchanged. In Table VII the hexobarbital half-lives have been compared for the volunteers who received the drug more than two times with interims of one week or more. It appears that if the interim between the consecutive experiments is longer than one week, then real differences in half-life may become apparent.

CONCLUSIONS

From the pharmacokinetic point of view, hexobarbital should be regarded as a suitable compound for hypnotic drug therapy. Due to its short half-life, the risk of residual effects the following morning is minimal. When hexobarbital is taken each night, no accumulation occurs.

For patients who have difficulty getting to sleep, a satisfactory therapy may be achieved by hexobarbital sodium (capsule VI). The rapid absorption of a 300 - 600 mg dose is expected to induce sleep within 30 min, assuming a minimal effective plasma level of 2 - 4 mg/l. If the difficulty involves the maintenance of sleep, then hexobarbital free acid in the form of capsule I or the EVIPAN tablets could be used. The minimal effective level may be reached 1 - 2 h after drug intake and the concentration should remain above this level for a few hours. Thus, a dose of 600 mg may be adequate. Obviously, hexobarbital free acid should not be used for the treatment of difficulties getting to sleep. Finally, the suppositories containing hexobarbital or hexobarbital sodium could be used in sedative drug therapy. During 4 - 7 hours the plasma level remains quite constant, which possible causes a constant degree of sedation. This must be regarded as beneficial in sedative therapy.

It should be emphasized that the use of hexobarbital in hypnotic and sedative drug therapy is not primarily advocated; rather, the compound has been used as a tool to illustrate how the principles of biopharmacy may be applied for the design of rational drug products. It becomes evident from the present investigation that the same active drug substance, incorporated in different pharmaceutical formulations, may serve different therapeutical goals. Furthermore it should be emphasized that the pharmacokinetic properties of a drug such as hexobarbital, in principle, may be favourable with respect to rational hypnotic drug therapy.

SUMMARY

Intravenous administration

The pharmacokinetics of hexobarbital were studied in fourteen healthy volunteers. Plasma levels were determined during and after a 30 or 60 min zero-order i.v. infusion of hexobarbital sodium (7.23 - 8.82 mg/kg). Hexobarbital kinetics were suitably described by assuming that the body exhibited two distinct compartments. The plasma concentrations were fitted according to the post-infusion equation and the parameters intrinsic to the two-compartment open model were estimated. The elimination half-lives varied considerably for the fourteen individuals (160 - 441 min, with a mean value of 261 min). This variation was explained mainly by the different metabolic clearance constants of the compound (123 - 360 ml/min). The apparent volume of distribution was relatively constant (1.10 $\pm$ 0.12 l/kg). Adipose tissue did not play a major role in hexobarbital elimination. The excretion of unchanged drug and of 3-keto-hexobarbital in urine was measured for five volunteers; less than 0.5% of the administered dose was excreted as unchanged hexobarbital and 29 - 63% as 3-keto-hexobarbital.

Oral and rectal administration

The absorption rate of hexobarbital and hexobarbital sodium from different dosage forms was studied after oral and rectal administration. Seven dosage forms were used in this investigation; their composition is outlined in Table III. Each preparation was administered to at least six healthy volunteers, who had fasted overnight. The administered dose was 600 mg hexobarbital or 600 mg hexobarbital sodium. Hexobarbital plasma concentrations were measured at frequent intervals, usually until 4 h after drug administration. Absorption with the dosage forms containing the sodium salt occurred more rapidly than with the preparations containing the free acid. For example the mean peak concentration with capsule VI (sodium salt), was reached after 50 min, as compared with more than two hours for the oral free acid preparations. Statistically significant differences were found for C_{max} and t_{max} between capsule VI and the oral free acid preparations. Differences among the latter and also among the capsules V and VI were not significant. Relative bioavailabilities of the oral preparations were estimated by comparing the average areas under the plasma concentration curves and taking capsule VI as the reference dosage form (100%). The bioavailability of capsule III was found to be substantially lower (63%) than that of the other preparations (86 - 89%). After rectal administration of hexobarbital or hexobarbital sodium, the plasma concentrations during the first 4 h remained much lower than after oral administration. Absorption was not complete after 4 h and when the concentrations were followed for longer time periods, it was found that the levels remained constant (around 2 mg/l) for several hours. Bioavailability, relative to capsule VI, was 40 - 60%. When a dose of 100 mg, instead of 600 mg, hexobarbital sodium was administered rectally, bioavailability increased per unit of dose, which indicated that the extent of absorption was related to the dosage.

A substantial variation in the absorption profile of the volunteers was apparent for each dosage form. Some additional experiments revealed that the absorption of

the same preparation in the same volunteer also was subject to considerable variation. However, for the same volunteer the hexobarbital half-lives, determined at different occasions, were relatively constant. Also, when hexobarbital was administered each night, for a period of 9 days, the half-life of the drug prior to and after the experiment remained constant.

As a result of these pharmacokinetic studies hexobarbital should be regarded as a suitable compound in hypnotic drug therapy. Due to its short half-life the risk of residual effects is minimal. Hexobarbital sodium may be used for a rapid induction of sleep, hexobarbital free acid for sleep maintenance and the suppositories for sedative drug therapy.

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CHAPTER 2

(+)- AND (—)-HEXOBARBITAL

A. PHARMACOKINETICS IN MAN

B. PHARMACOKINETICS IN RATS AND IN THE ISOLATED PERFUSED RAT LIVER

A. PHARMACOKINETICS IN MAN

INTRODUCTION

Early investigations (Hsueh and Marvel, 1928; Kleiderer and Shonle, 1934) did not reveal any real differences in anaesthetic potency between the optical antipodes of barbituric acid derivatives. In recent years, however, such differences have become evident in animal studies, e.g. secobarbital (Gibson et al., 1959; Haley and Gidley, 1970a), methylphenobarbital (Büch et al., 1968) and pentobarbital (Büch et al., 1969). Christensen and Lee (1973) have studied the anaesthetic activity and acute toxicity of the enantiomers of pentobarbital, secobarbital, thiopental and thiamylal. The S(—)-isomer was found in every case to be significantly more toxic and potent than the R(+)-isomer. Furthermore Downes et al. (1970) found that the (+)-isomer of 5-(1,3-dimethylbutyl)-5-ethyl barbituric acid caused tonic extensor seizures preceded by brief tonic flexion. In contrast, the (—)-isomer induced preanaesthetic excitation without tonic seizures and antagonized the seizure activity of the (+)-isomer. Similar findings on these compounds were reported by Sitsen and Fresen (1974).

The observations above-mentioned suggest that considerable differences in pharmacological respect may exist between the enantiomers of barbiturates. Knabe and Kräuter (1965) have described a procedure for the separation of the optical antipodes of hexobarbital. Wahlström (1966) and Wahlström et al. (1970) showed that with (—)-hexobarbital a higher total dose was required to produce anaesthesia in rats than with (+)-hexobarbital. Rummel et al. (1967) and Furner, McCarthy et al. (1969) noted that a longer sleeping time was in-

duced after (+)-hexobarbital administration to rats, whereas the same authors found that the (+)-isomer was metabolized more rapidly. In the second part of this Chapter the pharmacokinetics of the hexobarbital antipodes in rats will be described in detail and the differences in anaesthetic potency will be discussed.

Since no data were available on the differences in potency or rate of metabolism of the enantiomers of any barbiturate in man, it was considered of interest to study these aspects of the hexobarbital antipodes in man. For this purpose the enantiomers as well as racemic hexobarbital were administered to the same healthy volunteers. The central depressant symptoms were recorded and from the plasma concentration curves the elimination half-lives were determined (Breimer and van Rossum, 1973). In addition, the urinary excretion of unchanged hexobarbital and 3-keto-hexobarbital were measured.

MATERIALS AND METHODS

Samples of the hexobarbital optical antipodes were supplied by Prof. Dr. J. Knabe, Saarbrücken, GFR and by BAYER AG (Prof. Dr. G. Kroneberg), which are greatfully acknowledged. The preparation of further amounts of the antipodes was carried out by Mr. van Gemert according to the method of Knabe and Kräuter (1965). The specifications of the compounds were: (+)-hexobarbital $[\alpha]_D^{20} = +11.4°$ (ethanol), m.p. 153 - 154°; (—)-hexobarbital $[\alpha]_D^{20} = -11.2°$ (ethanol), m.p. 152 - 153.5°. Racemic hexobarbital (m.p. 142 - 144°) was obtained from Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands.

Five healthy male volunteers, ranging in age from 20 - 25 years and in bodyweight from 68 to 79 kg, participated in the study. They had received no regular medication during the 4 weeks preceding initiation of the experiments. The trials began in the morning (9 a.m.), after the subjects had fasted overnight and 400 mg of one of the compounds in powder form was administered orally together with 200 ml water. Initially they were asked to remain in an upright position for at least 15 min and then to lie down for at least 3.5 h. No food, fluid or tobacco was allowed for 3.5 h after drug administration. Blood samples were taken from a forearm vein at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 7.0, 9.0 and 11 or 12 h after intake of drug. Plasma concentrations were determined by gas chromatography with nitrogen selective detection (Chapter 2, Section II). Each volunteer received all three compounds with an interval of at least two weeks between two consecutive trials. The sequence of administration of the drugs was randomly chosen.

Two of the volunteers collected their urine for 24 h each time after administration of one of the compounds. Hexobarbital and 3-keto-hexobarbital concentration in urine was determined by gas chromatography as described in Chapter 2 and 3 of Section II.

RESULTS AND DISCUSSION

Half-lives and metabolic clearance of (+)- and (—)-hexobarbital

The mean hexobarbital plasma levels following oral administration of 400 mg (+)-, (—)- and (±)-hexobarbital to five healthy volunteers are presented in Table I. In Fig. 1 the three plasma concentration curves of one volunteer are shown. It is evident that the plasma disappearance rate of (—)-hexobarbital was much greater than that for (+)-hexobarbital. Elimination occurred according to a first-order process and from the descending part of the curves the elimination half-lives have been deduced. These are given in Table II for the five volunteers. In man the half-life of (—)-hexobarbital is more than three times shorter than of (+)-hexobarbital. In rats the reverse has been observed, the half-life of the (—)-isomer being about 2.5 times longer than that of the (+)-isomer (Breimer and van Rossum, 1974; part B of this Chapter). The reason for this species difference is not clear. In in vitro experiments with rat liver microsomes Degkwitz et al. (1970) and Furner, McCarthy et al. (1969)

TABLE I. Mean plasma concentrations (mg/l) of hexobarbital after oral administration of (+)-, (—)- and (±)-hexobarbital to five volunteers.

Time (h)	(—)	(+)	(±)
0.5	0.83 (0.32-2.12)*	1.20 (0.41-1.69)	0.55 (0.16-0.95)
1	1.47 (0.48-2.60)	3.49 (2.22-4.04)	1.78 (0.86-2.69)
1.5	1.73 (0.56-3.38)	4.15 (3.70-4.77)	2.63 (1.35-4.08)
2	2.71 (1.42-3.92)	3.96 (3.30-4.79)	2.51 (1.71-3.24)
3	1.63 (0.88-2.28)	3.39 (3.09-4.10)	2.38 (1.88-3.06)
4	0.85 (0.54-1.22)	2.71 (2.39-2.87)	1.85 (1.63-2.08)
5	0.59 (0.28-0.96)	2.32 (1.94-2.45)	1.59 (1.44-1.88)
7	0.28 (0.11-0.42)	1.93 (1.38-2.33)	1.18 (0.90-1.55)
9	0.10 (0.04-0.18)	1.24 (0.96-1.49)	0.87 (0.66-1.32)
12	—	0.84 (0.58-1.29)	0.50 (0.25-0.71)

* Concentration range in parentheses.

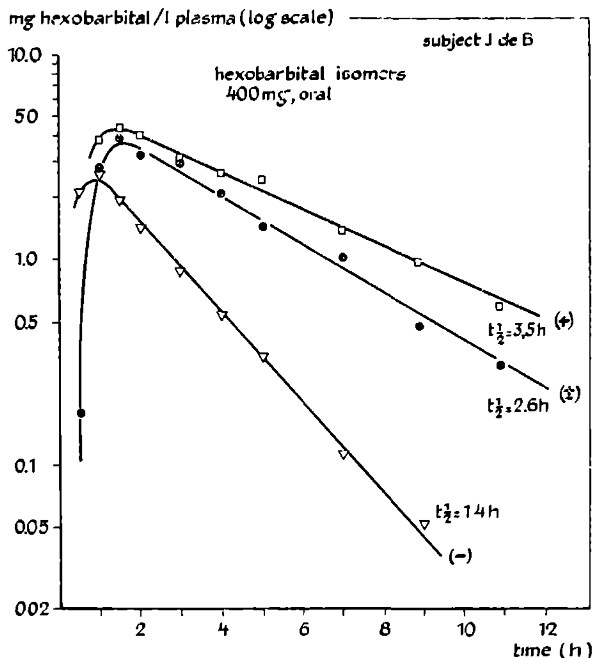

FIG. 1. Hexobarbital plasma concentration curves (semi-logarithmic scale) after oral administration of (+)-, (—)- and (±)-hexobarbital (400 mg) to the same volunteer.

showed a comparable affinity of the two isomers for the mixed function oxygenase system (cytochrome P-450), but a higher rate of conversion by this system for the (+)-isomer. Similar experiments with human liver homogenates are in progress in order to explain this reversed situation in man. It is interesting to note that a species difference for the elimination of the pentobarbital optical antipodes also has been found. Büch et al. (1969) found that (—)-pentobarbital was more rapidly inactivated in rats than (+)-pentobarbital, whereas Mark et al. (1971) observed the reverse in dogs.

Since the hexobarbital enantiomers have identical physico-chemical properties, except optical rotation, there is no reason to assume that a significant difference in apparent volume of distribution for the isomers in the same subject will exist. Moreover it was shown that, at least at higher concentrations, there exist no significant differences in protein binding between (+)- and (—)-hexobarbital (part B of this Chapter). Therefore, the differences in half-life must be explained on basis of a difference in the rate of metabolism. The metabolic

TABLE II. Plasma half-lives (h) of (+)-, (—)- and (±)-hexobarbital after oral administration to five volunteers.

Subject	Compounds			Ratio (+) / (—)
	(±)	(+)	(—)	
W.K.	3.8	4.0	1.3	3.1
B.R.	4.3	6.3	1.6	3.9
J. de B.	2.6	3.5	1.4	2.5
F.I.	4.0	4.3	1.3	3.3
W.D.	5.0	4.7	1.6	2.9
mean	4.0	4.6	1.4	3.2

clearance constant for (—)-hexobarbital in man must be approximately three times greater than for (+)-hexobarbital. The exact values for this parameter cannot be estimated from the present experiments, since the bioavailability after oral administration remains an uncertain factor. However, after intravenous infusion of racemic hexobarbital sodium into man the metabolic clearance constant for the racemate was found to be approximately 260 ml/min (Chapter 1, Section III). This means that the value of this parameter for (—)-hexobarbital may be in the range of 650 - 750 ml/min, because the half-life is almost a factor three shorter than that of the racemate. Rowland (1972a) has shown that if hepatic clearance of a drug is large, the amount of drug that reaches the general circulation intact may be substantially lower than the dose administered orally. After absorption a relatively large fraction of the amount of drug that reaches the portal blood may be metabolized during the first passage through the liver. It may be expected that this so-called "first-pass effect" applies in the case of (—)-hexobarbital, since its clearance is relatively high in relation to blood flow through the liver (1500 - 2000 ml/min). For any linear pharmacokinetic model the following applies:

$$Q = k_{el} \cdot \int_{0}^{\infty} C_p dt = k_{el} \cdot AUC$$

where Q is the amount of drug that reaches the general circulation intact, k_{el} is the clearance constant, C_p is the plasma concentration and AUC is the area under the plasma concentration curve from $t = 0$ to $t = \infty$. The average area for (—)-hexobarbital was determined and found to be 7.8 mg.h/l. If it is as-

sumed that the average k_{el} for the five volunteers was 700 ml/min (42 l/h), then Q = 328 mg. If absorption was complete, it follows that about 18% of the (—)-isomer was metabolized during the first passage through the liver. This is less than expected on basis of the equation derived by Rowland (1972a) for the unmetabolized fraction Θ:

$$\Theta = 1 - \frac{\text{hepatic clearance}}{\text{liver blood flow}}$$

When the liver blood flow is assumed to be about 1500 - 2000 ml/min theoretically 30 - 50% should have been metabolized during the first passage through the liver. The discrepancy between this value and the experimental one indicates that the situation for (—)-hexobarbital is probably more complicated than expressed by Rowland's formula. Further information will be obtained by administering the substance i.v. so that a more accurate clearance value can be determined.

The average AUC for (+)-hexobarbital was also determined (30.7 mg.h/l). Assuming an average clearance constant of 218 ml/min (the value for the (—)-isomer divided by the half-life ratio) Q is found to be 402 mg. This may indicate that the absorption of (+)-hexobarbital was complete and, in addition, that there is no notable "first-pass effect" with this isomer.

The elimination phase of racemic hexobarbital

Theoretically, a straight line for the elimination phase of racemic hexobarbital should not be obtained, since the two different time constants for the antipodes are underlying this process. While the (—)-isomer contributes to the elimination in the first few hours, this is also the time during which absorption takes place, the pure elimination phase having not yet been reached. The calculated half-lives are based on the points 3 - 4 h after administration and it can be deduced from the concentrations in Table I that in the period between 4 and 12 h the plasma concentration of the (—)-isomer, when compared with the (+)-isomer is so low that it makes little contribution to the curve of the racemate. In Fig. 2 the theoretical line for the racemic mixture has been calculated, which is based upon the experiments of Fig. 1. The experimental lines of (+)- and (—)-hexobarbital were plotted and the curve for the racemate was calculated, taking at each time the sum of half the concentration of each isomer. It may be observed that the theoretical elimination line obtained hardly deviates from linearity. In practice the scatter in the experimental data will not distinguish between

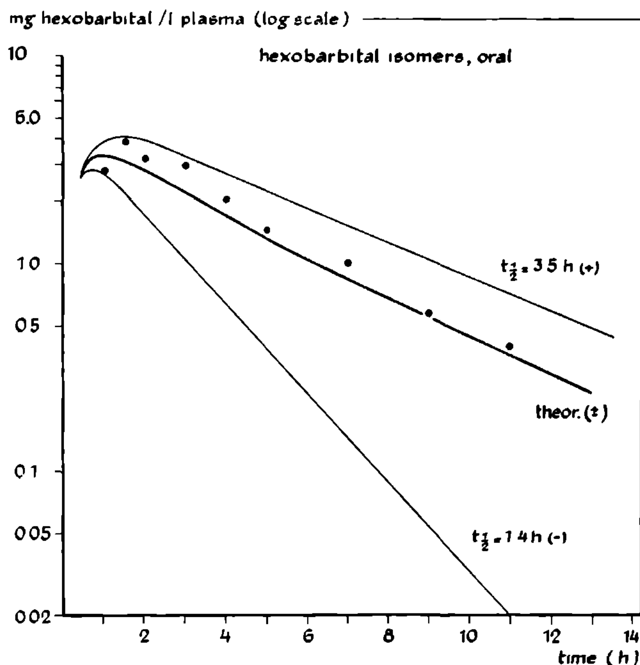

FIG. 2. Theoretical plasma concentration curve for racemic hexobarbital, based upon the experimental curves for (+)- and (—)-hexobarbital (subject J. de B., Fig. 1). The theoretical line was obtained by taking at each time the sum of half the concentration of (+)-hexobarbital and half the concentration of (—)-hexobarbital. Note that this line hardly deviates from linearity, due to the small contribution of the (—)-isomer. The experimental plasma concentrations of racemic hexobarbital are slightly higher than expected, which may be caused by a slightly varying bioavailability.

the two distinct phases in the elimination process of the racemate. The concentrations fit a straight line and in most instances a slightly shorter half-life is estimated for the racemate, than that estimated for the (+)-isomer (Table II). Also, after a 30 min or 60 min zero-order i.v. infusion differentiation between the two elimination time constants was impossible, as was discussed in Chapter 1 of this Section. The question arose as to whether, after rapid i.v. administration, such a differentiation could be made. In preliminary experiments 300 mg of racemic hexobarbital sodium was administered by rapid i.v. infusion to two healthy human volunteers. The results of one experiment are given in Fig. 3. After perusal of the curve three distinct phases could be distinguished: a rapid distribution phase corresponding to a half-life of 14 min, a slower phase with

a half-life of 1.2 h and finally a phase with a half-life of 5.7 h. It seems likely that the two latter phases are due to the different rates of elimination of the antipodes, since these half-lives are in the same order of magnitude as found after oral administration of the individual isomers. Surprisingly, in the second volunteer such a distinction could not be made, only a distribution phase and a single first-order elimination phase became apparent. Further experiments of rapid i.v. administration are being done in order to determine the critical factors that play a role in such complex elimination patterns.

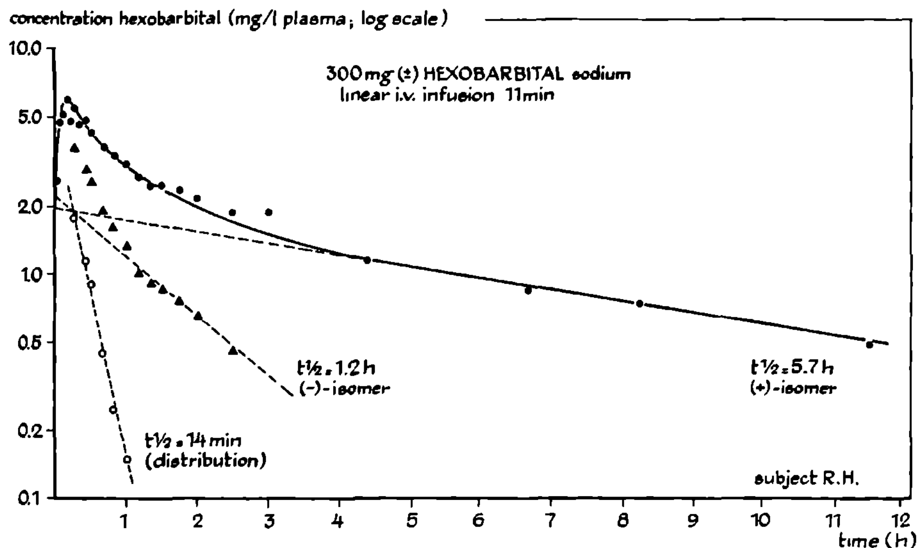

FIG. 3. Hexobarbital plasma concentration curve after rapid zero-order i.v. infusion of racemic hexobarbital sodium into a healthy volunteer. By „peeling-off" the post-infusion curve three phases can be distinguished. It is likely that the half-lives of 1.2 h and 5.7 h correspond to the elimination of (—)-hexobarbital and (+)-hexobarbital respectively.

Urinary excretion of unchanged hexobarbital and 3-keto-hexobarbital

Since the rate of metabolism of the hexobarbital antipodes in man exhibits considerable differences, it was considered of interest to obtain additional information on the metabolic fate of these compounds. Therefore the renal excretion of unchanged hexobarbital and 3-keto-hexobarbital was determined for

two volunteers who received the racemic mixture and the two isomers, subsequently. No quantitative data are available in the literature on the excretion of hexobarbital or metabolites. Previously, Tsukamoto et al. (1957) and Frey et al. (1959) identified 3-keto-hexobarbital in human urine after administration of racemic hexobarbital. In Fig. 4 an example is given of the renal excretion curves obtained. The cumulative urinary excretion (0 - 24 h) of unchanged drug and 3-keto-hexobarbital is summarized in Table III. Hexobarbital is hardly

TABLE III. Cumulative renal excretion of hexobarbital and 3-keto-hexobarbital after oral administration of (+)-, (—)- and (±)-hexobarbital (400 mg).

Subject	Drug administered	Hexobarbital		3-keto-hexobarbital	
		mg	% of dose	mg	% of dose
J. de B.	(+)	2.02	0.51	65	16
	(—)	0.23	0.06	274	65
	(±)	0.28	0.07	164	39
W.K.	(+)	2.28	0.57	60	15
	(—)	0.07	0.02	161	38
	(±)	0.56	0.14	105	26

excreted unchanged into the urine. (—)-Hexobarbital appears to be metabolized substantially more to 3-keto-hexobarbital than (+)-hexobarbital. Whether the quantitative differences in metabolism between the isomers is caused by stereoselective metabolism of hexobarbital itself, or that the intermediate 3-hydroxyhexobarbital (Bush and Weller, 1972) is involved, is uncertain and requires further investigation. In all instances 3-keto-hexobarbital was rapidly eliminated, its urinary half-life varied from 3 - 5 h. This may be regarded as a true elimination half-life, since also after (—)-hexobarbital administration the half-life was in the above-mentioned range. 3-Keto-hexobarbital does not possess hypnotic properties (Bush et al., 1953; Yoshimura, 1958).

Hypnotic potency of the enantiomers in man

With respect to hypnotic potency in man, the subjects experienced severe drowsiness which lasted several hours after 400 mg (+)-hexobarbital, whereas

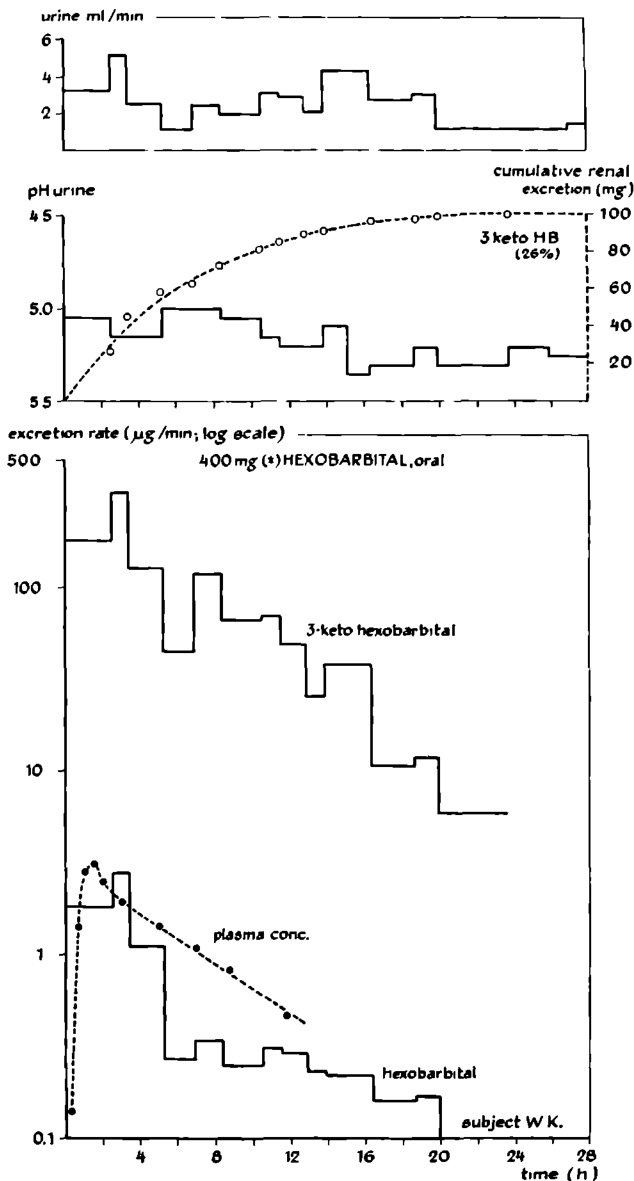

FIG. 4. Urine flow, urine pH, cumulative renal excretion of 3-keto-hexobarbital, renal excretion rate of 3-keto-hexobarbital and hexobarbital, and plasma concentration (mg/l) of hexobarbital after oral administration of 400 mg racemic hexobarbital. Only a very small fraction (0.14%) of the administered dose was excreted unchanged into the urine.

after (—)-hexobarbital no noticeable effect was experienced. After administration of the racemic mixture moderate feelings of lethargy became apparent, which were of relatively short duration. Although it seems likely that in man the (+)-isomer has a greater activity than the (—)-isomer, as is the case in rat, one must be aware of the fact that the above observations might also be explained on the basis of the differences in disappearance rate of the two isomers in man.

B. PHARMACOKINETICS IN RATS AND IN THE ISOLATED PERFUSED RAT LIVER

INTRODUCTION

Hexobarbital possesses a chiral centre at C_5 due to the presence of a methylgroup on one of the nitrogen atoms in the barbituric acid ring. Since Knabe and Kräuter (1965) described a procedure for the separation of the optical antipodes, some reports on the activity and on the fate of the isomers in animals have appeared. Wahlström (1966) and Wahlström et al. (1970) showed that with (—)-hexobarbital a higher total dose was required to produce anaesthesia in rats than with (+)-hexobarbital. Rummel et al. (1967) and Furner, Mc Carthy et al. (1969) noted a longer sleeping time in rats after i.p. injection of (+)-hexobarbital than after (—)-hexobarbital and the latency time appeared also to be shorter with the (+)-isomer. In addition, Furner, Mc Carthy et al. (1969) found that (+)-hexobarbital was metabolized more rapidly in vitro by rat liver microsomes and had a shorter half-life in vivo. Degkwitz et al. (1969) studied the binding of the two antipodes to cytochrome P-450 and reported differences in the magnitude of the spectral change. A detailed investigation on the kinetic differences in the microsomal metabolism of the isomers and of the racemate in rats, rabbits and mice was carried out by Mc Carthy and Stitzel (1971). In all three species (—)-hexobarbital is metabolized at a slower rate than (+)-hexobarbital. In contrast to these results it was found by us, studying the pharmacokinetics of the two antipodes in healthy human subjects, that the half-life of the (—)-isomer is approximately three times shorter than the half-life of the (+)-isomer, suggesting that in man the latter isomer is slower metabolized (Breimer and van Rossum, 1973).

Because of the reported differences in rate of metabolism and possibly also in distribution behaviour (Rummel et al. 1967) of the enantiomers in rats, it was considered to be of interest to study the pharmacokinetics of the compounds in

more detail. We decided to compare the pharmacokinetics of the two compounds in the same individual rat, since in preliminary experiments with racemic hexobarbital it was found that the half-life did not differ more than 20 per cent when determined in the same rat at interims of 1 week. Apparently the intraindividual variability is not so great, whereas Alvarez (1971b), studying the pharmacokinetics of barbiturates in individual rats, noted a substantial interindividual variability.

A comparison is made of some important pharmacokinetic parameters such as the total body clearance constant (k_{el}) and the apparent volume of distribution (V_f), which are the main factors governing the overall disappearance rate of a drug from the body (van Rossum, 1971). These and other parameters, determined in the same animal, will give more information about stereoselective metabolism and distribution of hexobarbital. The binding to serum albumin was also determined to see whether or not stereospecific interaction exists, which could contribute to stereoselective distribution. Furthermore the data in vivo are compared to those obtained in vitro in the isolated perfused rat liver.
Finally the pharmacokinetic results are discussed with respect to possible differences in anaesthetic potency between the enantiomers.

MATERIALS AND METHODS

Drugs

The enantiomers of hexobarbital were prepared according to the method of Knabe and Kräuter (1965);
(+)-hexobarbital: $[\alpha]_D^{20} = +11.4°$ (ethanol), m.p. 153° - 154°;
(—)-hexobarbital: $[\alpha]_D^{20} = -11.2°$ (ethanol), m.p.152° - 153.5°.
Shortly before use each compound was dissolved in an equimolar quantity of sodium hydroxide and diluted with 0.9% sodium chloride solution to a concentration of 30 mg barbiturate per ml.

In vivo experiments

Male Wistar rats, weighing approximately 200 g, were used for the in vivo experiments. They were maintained at standard laboratory diet and fasted overnight before the experiments. Water could be taken ad libitum. The animals were injected i.v. in the dorsal vein of the penis at 9 o'clock a.m. No anaesthesia was applied and during the injection procedure the animals were kept in such a cage, that moving was impossible. The mean injection time was about 10 sec.

The injection volume dit not exceed 0.5 ml. Sleeping times were determined as the time elapsed from the loss till the regaining of the righting reflex. Blood samples (0.2 - 0.5 ml) were taken at several times after injection through orbital puncture, using heparinized micro-hematocrit tubes. The blood was collected in tubes containing a minute amount of heparin. Both isomers were administered to the same animal in random order with interims of one week. The dose of the compounds was always the same per animal and they are given in Table IV.

The isolated perfused rat liver

Male Wistar rats (the same strain as used in the in vivo experiments), weighing approximately 200 g, were used for these in vitro experiments. The perfusion technique was modified after the method described by Miller et al. (1951). The surgical procedure and perfusion apparatus have been outlined by Northrop and Parks (1964). The perfusion fluid was equilibrated with oxygen, containing 5% CO_2 and was supplied to the isolated liver by a portal vein cannula. Bile was collected with a bile duct catheter. The temperature in the perfusion chamber was kept at 38° ± 0.2° C. An artificial perfusion liquid was used according to Schimassek (1962), consisting of electrolytes, human erythrocytes, bovine albumin and glucose. The preparation of this fluid was carried out under aseptic conditions. The volume of the perfusate was 100 ml. The routine criteria for the performance of the liver were: bile production, perfusate flow through the liver (15 - 20 ml/min) and macroscopic appearance of the liver. After an equilibration time of 30 min, 5 or 10 mg of one of the hexobarbital antipodes was introduced into the perfusion fluid, while 0.5 ml samples were taken at intervals of 5 min. After 45 minutes the other isomer was added to the perfusate and again samples were taken every 5 minutes.

Determination of hexobarbital blood concentrations

Hexobarbital blood concentrations were determined by means of gas chromatography, according to a modified method described by Jain and Kirk (1967). To the blood sample (0.2 - 0.5 ml) 10 μg of pentobarbital dissolved in 0.1 ml ethanol was added as internal standard.

Extraction was carried out twice with 2 ml ether/acetone (1 : 1) (both analytical-reagent grade) on the whirlmixer. The combined extracts were evaporated to dryness and the residue was redissolved in a small volume (50 μl - 100 μl) of chloroform or methanol. Of this solution 2 - 5 μl was injected into a Hewlett & Packard, Model 402, gaschromatograph with flame ionization detection. Glass columns (1.8 m x 3 mm i.d.) were packed with 5% SE-30 on Diatoport S.

60 - 80 mesh. The injection block was kept at 230° C, the column at 200° C and the detector at 250° C. Gas flow rates were: carrier (nitrogen) 30 ml/min, air 400 ml/min and hydrogen 40 ml/min. Hexobarbital concentrations were calculated by measuring the ratio of the area of the hexobarbital peak to the area of the internal standard peak and multiplying by a factor derived from a calibration curve.

Protein binding studies

The binding of the two isomers and of racemic hexobarbital to bovine serum albumin was investigated by equilibrium dialysis. This was carried out in cylindrical metal cells (i.d. 2.5 cm), separated by a semipermeable membrane (cellulose acetate, AKU, Arnhem). Both cells contained equal volumes (2.0 ml) of phosphate buffer (0.1 M), pH 7.4; in addition one of the cells contained 3% demineralized bovine serum albumin (Povite, Amsterdam) and one of the compounds, at start concentrations of 0.25, 0.125 or 0.063 mM. The cells were placed in a water bath at 37° C and rotated at 1 r.p.m. for 24 hours (this equilibrium period was selected in preliminary experiments). The hexobarbital concentrations were measured in both dialysis chambers by extracting 0.5 ml aliquots with 5 ml ether and further using the gas chromatographic procedure as described for the determination of hexobarbital in blood. Occasionally the albumin content after dialysis was checked by measuring UV-absorption at 279 nm. In the albumin-containing compartment the total hexobarbital concentration (C_t) measured, equals the albumin bound concentration plus the unbound or free concentration (C_f). In the equilibrium state the concentration measured in the protein free compartment equals C_f. The percentage of binding is calculated from $\frac{C_t - C_f}{C_t} \times 100$.

RESULTS

In vivo

I.v. administration of either antipode generally resulted in the loss of the righting reflex within 10 sec (the average injection time). This means that there is no measurable difference in onset of anaesthetic action between the two compounds after i.v. injection. However, the times elapsed until the regaining of the righting reflex (sleeping time) were found to be quite different. All animals

TABLE IV. Sleeping times and blood concentrations at the moment of awakening of (+)- and (—)- hexobarbital in the same rat.

Rat no.	Dose (mg)		Sleeping time (min)		Conc. (μg/ml)	
	(+)	(—)	(+)	(—)	(+)	(—)
1	15	15	38	3	14	64
2	15	15	29	15	18	62
3	15	15	37	3	17	72
4	10	10	21	5	18	48
5	15	15	22	0	18	—
6	12.5	12.5	23	2	12	37
7	12.5	12.5	12	4	12	45
8	12.5	12.5	19	0	13	—
mean ± S.D.					15 ± 2.8*	55 ± 13.4*

* Significantly different at $P < 0.005$ (Student's t-test).

slept much longer after receiving (+)-, than after receiving (—)-hexobarbital (Table IV). There is a 1.5- to 12-fold difference in sleeping time in the various animals. After receiving (—)-hexobarbital only a very short loss of the righting reflex was observed, while even in two animals no such an effect occurred at all. A difference in behaviour was noted after regaining the righting reflex; after (+)-hexobarbital the animals were quite normal in their motor activity compared to uninjected rats, whereas after (—)-hexobarbital their spontaneous movements were much diminished for about 30 to 60 minutes. Similar observations have been reported by Furner, Mc Carthy et al. (1969). The hexobarbital blood concentrations at the moment of awakening are also given in Table IV. They have been deduced from the total blood concentration-time curves. The concentration at the moment of awakening in the same rat after injection of (+)-hexobarbital is three to four times lower than after (—)-hexobarbital. It is also evident that these concentrations for the (+)-antipode fall within a narrow range, with a mean value of 15 μg/ml blood. This value for the (—)-isomer was 55 μg/ml, however with a substantial standard deviation. These mean values can approximately be considered as the average minimum effective levels exerting an anaesthetic effect in rats. Furner, Gram and Stitzel (1969) determined this level for racemic hexobarbital in different rat strains and they found for Wistar rats 36 μg/ml blood, which is halfway between the two values for (+)- and (—)-hexobarbital.

The blood concentrations were determined at several time intervals after taking small blood samples by orbital punction. It was attempted to get at least three samples during the first 5 min after injection, as in that period there is a rapid fall in blood concentration mainly due to distribution of the drug from the blood into the tissues. In the next 50 min at least five more samples were taken. Fig. 5 shows a typical example of blood concentration-time curves of

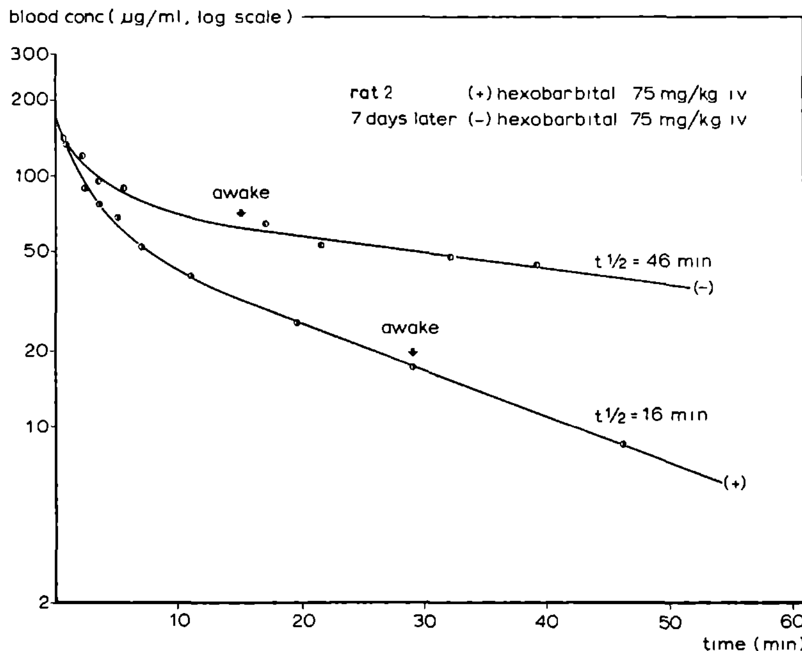

FIG. 5. Hexobarbital blood concentration curves after i.v. administration of (+)- and (—)-hexobarbital to the same rat.

the two antipodes in the same rat on semi-logarithmic scale. Apparently the time course of these curves can be described by the sum of two exponential equations, which is characteristic for the so-called two-compartment open model (Riegelman et al., 1968). The second phase represents the overall elimination process of the drug in the body. The blood concentration can be described by the following equation (van Rossum, 1971):

$$C_p = A_1 \cdot e^{-t/\tau_1} + A_2 \cdot e^{-t/\tau_2}$$

in which C_p is the blood concentration at time t and A_1 and A_2 are the zero-

time concentration intercepts. A_1 and A_2 are associated to the small (τ_1) and to the large (τ_2) time constant respectively. These four parameters can easily be deduced from the experimental curve and then various kinetic parameters according to the two-compartment open model after rapid i.v. infusion can be calculated as outlined in the Appendix. The time constants τ_1 and τ_2 depend on the volume of the first compartment and that of the second compartment, as well as on the distribution clearance constants (k_{12} and k_{21}) and the total body elimination clearance constant (k_{el}). The time constant of the slowest phase is simply related to the biological half-life ($t_{1/2}$):

$$t_{1/2} = 0.69 \cdot \tau_2.$$

which is a general measure for the overall elimination rate of a drug from the body. In Table V the half-lives of (+)- and (—)-hexobarbital are given, as well

TABLE V. Half-lives of (+)- and (—)-hexobarbital in the same rat.

Rat no.	$t_{1/2}$ (+) (min)	$t_{1/2}$ (—) min	Ratio $\frac{t_{1/2}(-)}{t_{1/2}(+)}$
1	14	30	2.2
2	16	46	2.9
3	26	32	1.2
4	21	57	2.7
5	23	73	3.2
6	14	34	2.4
7	16	36	2.3
8	17	46	2.7
mean ± S.D.	18.4 ± 4.4*	44.3 ± 14.7*	2.5 ± 0.6

* Significantly different at $P < 0.001$ (Student's t-test).

as the ratio $t_{1/2}$ (—)/$t_{1/2}$ (+). The half-life of (—)-hexobarbital is two to three times longer than of (+)-hexobarbital, with a mean ratio of 2.5. The differences are clear in the individual animals as well as expressed in the mean values. These mean values of 44.3 and 18.4 min are in close agreement with those measured by Furner, Mc Carthy et al. (1969), who found 48.2 and 17.4 min respectively in male rats. It is evident that the (+)-isomer disappears

faster from the blood than the (—)-isomer. Calculation of the total body clearance constant revealed that for this parameter there is also a 2- to 3-fold difference between the two compounds (Table VII). The apparent volumes of distribution did not differ more than a factor 1.5 (Table VII), neither did the volume of the first compartment (V_1) nor did the distribution rate constants. So the only rate determining parameter which is essentially different for the antipodes is the total body elimination clearance constant. As elimination of hexobarbital in rats takes place by metabolism only (less than 1 per cent of unchanged drug was found in bile and urine), this means that the rate of metabolism of (+)-hexobarbital is 2 to 3 times higher than of (—)-hexobarbital in vivo. So the difference in half-life is directly related to a difference in metabolic clearance and not to a difference in volume of distribution.

The isolated perfused rat liver

The isolated perfused rat liver preparation is a useful in vitro tool for the study of drug metabolism (e.g. Von Bahr et al., 1970). Alvarez (1971a) conducted a comparative study of barbiturate metabolism in the perfused liver and in vivo in rats. Good correspondence was found between clearance values in vitro and in vivo for the compounds eliminated by metabolism mainly.

To allow comparison, the kinetics of (+)- and (—)-hexobarbital further were investigated in the isolated perfused rat liver. Since metabolism of the two compounds is relatively fast, it was possible to study the fate of both compounds in the same liver. A 45 min interval was chosen between the introduction of one compound or the other into the perfusion fluid. When (—)-hexobarbital was added firstly, it was found by extrapolation that still 10 - 20% of this compound was present after 45 min. In that case the (+)-hexobarbital concentrations were corrected for the presence of (—)-hexobarbital, assuming that the rates of metabolism were not influenced by the presence of each other. Preliminary experiments revealed that the uptake of the two compounds by the liver in the perfusion system could be neglected. The distribution in the system is quite homogeneous and therefore it can be considered as one compartment. This is consistent with the results shown in Fig. 6, in which the blood concentration-time curve for the two antipodes in the same isolated rat liver system is shown on semi-log scale. Elimination occurs apparently according to a first-order process and the concentration of the drugs can be described by a mono-exponential equation (van Rossum, 1971):

$$C_p = C_0 \cdot e^{-t/\tau_{el}}$$

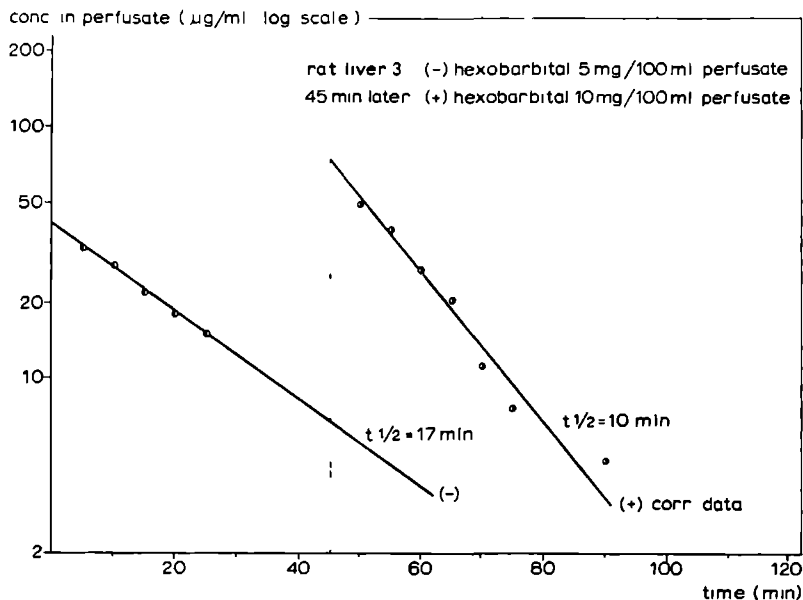

FIG 6 Hexobarbital liver perfusate concentration curves after adding 5 mg (—)-hexobarbital at zero time and 10 mg (+)-hexobarbital at 45 min to the perfusate of the same isolated liver The concentrations of (+)-hexobarbital were corrected for the presence of (—)-hexobarbital, assuming that the rate of metabolism was not influenced by the presence of each other

where C_p is the blood concentration (perfusion fluid concentration) at time t, C_0 is the zero-time concentration and τ_{el} is the elimination time constant. Elimination is considered as a clearance from a single compartment with a volume V_f, where

$$\tau_{el} = \frac{V_f}{k_{el}} \quad \text{or} \quad t_{1/2} = \frac{0.69 \cdot V_f}{k_{el}}$$

The half-life can easily be deduced from the experimental curve, whereas V_f equals Dose/C_0. The metabolic clearance constant can now be calculated. In Table VI the half-lives of the antipodes in the same perfused rat liver systems are given, as well as the ratio $t_{1/2}$ (—)/$t_{1/2}$ (+). The disappearance of (+)-hexobarbital is extremely rapid and there exists a 2- or 3-fold difference in half-life between the (+)- and the (—)-isomer. This is approximately the same ratio as

TABLE VI. Half-lives of (+)- and (—)-hexobarbital in the same isolated perfused rat liver.

Liver no.	$t_{1/2}$ (+) (min)	$t_{1/2}$ (—) (min)	Ratio $\frac{t_{1/2}(-)}{t_{1/2}(+)}$
1	11	28	2.5
2	7	10	1.4
3	10	17	1.7
4	11	23	2.1
5	9	22	2.4
6	6	21	3.5
mean ± S.D.	9.0 ± 2.2*	20.2 ± 6.1*	2.3 ± 0.8

* Significantly different at $P < 0.005$ (Student's t-test).

found in vivo, although the absolute values for the half-lives differ quite substantially.

As already mentioned, the rate of elimination of a drug is mainly dependent on the total body clearance and on the apparent volume of distribution. In Table VIII the apparent volumes of distribution of the isolated perfused liver systems are given. They are about the same for the two antipodes, but smaller than those in vivo. The clearance constants on the other hand (Table VIII) are almost equal to those in vivo. So the differences in half-life between the in vivo and the in vitro situation is due to a difference in volume of distribution. When comparing the kinetics of a drug in the isolated perfused liver system to the kinetics in vivo, the clearance is the meaningful parameter to look at. Alvarez (1971b) already did so for some barbiturates and Rowland (1972) paid attention to this point in reevaluating data by Von Bahr et al. (1970). Clearance is dependent on blood flow through the liver and the capacity of the liver to eliminate the drug (Rowland et al. 1973). Blood flow is the same for the two compounds and excretion into the bile of the unchanged compounds was less than 1 per cent. It can be concluded that in rats metabolism is a stereospecific process, since the clearance constants for the two antipodes show a 2- to 3-fold difference. Degkwitz et al. (1970) showed that the microsomal mixed function oxygenase system is involved in the metabolism of both antipodes in the rat and that the specific activity of this system was 1.6 times higher for (+)-hexobarbital than for (—)-hexobarbital. Approximately the same ratio was found by Furner, Mc Carthy et al. (1969) in hepatic microsomes of male rats. No sub-

TABLE VII. Pharmacokinetic parameters of (+)- and (—)-hexobarbital in rats.

Rat no.	1		2		3		4		5	
	(+)	(—)	(+)	(—)	(+)	(—)	(+)	(—)	(+)	(—)
Dose (mg/kg)	75	75	75	75	75	75	47.6	54.0	75	75
Weight (g)	200	200	200	200	200	200	210	185	200	200
A_1 (μg/ml)	101	85	89	75	136	168	17	39	94	109
A_2 (μg/ml)	86	57	60	77	38	45	39	45	37	37
τ_1 (min)	1.7	1.5	2.9	3.5	3.4	3.3	4.1	2.4	3.1	2.6
τ_2 (min)	20.3	43.2	23.4	67.0	37.4	46.0	30.2	82.1	33.3	105.1
V_1 (ml)	80	110	100	99	86	70	177	119	114	102
V_1/kg	400	550	500	495	430	350	844	641	570	510
V_f (ml)	150	238	182	177	231	211	229	211	270	352
V_f/kg	750	1190	900	885	1155	1055	1092	1142	1350	1760
k_{el} (ml/min)	7.8	5.8	9.0	2.8	7.9	5.7	8.0	2.6	9.8	3.6
k_{12} (ml/min)	19.4	37.2	13.4	11.9	12.3	11.4	9.4	21.1	17.6	26.1
r_{12} (1/min)	0.24	0.35	0.13	0.12	0.14	0.16	0.05	0.17	0.15	0.25
r_{21} (1/min)	0.30	0.28	0.16	0.15	0.08	0.08	0.18	0.23	0.11	0.10

TABLE VII continued.

Rat no.	6		7		8		Mean values ± S.D.	
	(+)	(—)	(+)	(—)	(+)	(—)	(+)	(—)
Dose	62.5	65.8	62.5	56.8	65.8	62.5	—	—
Weight	200	190	200	220	190	200	—	—
A_1	100	84	77	93	83	70	—	—
A_2	38	34	27	32	35	38	—	—
τ_1	0.8	1.2	1.0	0.7	1.6	2.3	2.3 ± 1.2	2.2 ± 1.0
τ_2	20.2	48.9	23.0	51.8	24.5	66.2	26.5 ± 6.4*	63.8 ± 21.3*
V_1	90	105	120	100	106	115	112 ± 32	103 ± 15
V_1/kg	450	555	600	562	557	575	544 ± 141	530 ± 85
V_t	270	327	387	361	270	291	249 ± 71	271 ± 71
V_t/kg	1350	1719	1835	1643	1420	1455	1232 ± 337	1356 ± 332
k_{el}	14.7	7.1	17.9	7.2	12.6	4.7	11.0 ± 3.7*	4.9 ± 1.8*
k_{12}	68.3	56.2	72.4	99.5	35.2	28.5	31.0 ± 26	36.5 ± 29
r_{12}	0.76	0.53	0.66	0.99	0.33	0.25	0.30 ±0.25	0.36 ± 0.29
r_{21}	0.38	0.25	0.29	0.38	0.21	0.16	0.22 ± 0.10	0.21 ± 0.10

* Significantly different at $P < 0.001$ (Student's t-test); all other values $P > 0.05$.

Liver no.	1 (+)	1 (—)	2 (+)	2 (—)	3 (+)	3 (—)	4 (+)	4 (—)
Dose (mg)	10	10	10	5	10	5	10	10
C_o (μg/ml)	75	72	90	41	69	40	78	96
τ_{el} (min)	15.8	40.3	10.1	14.4	14.4	24.5	15.8	33.1
V_f (ml)	133	139	111	122	146	123	128	104
k_{el} (ml/min)	8.6	3.4	11.0	8.6	10.1	5.0	8.1	3.1

TABLE VIII continued.

Liver no.	5 (+)	5 (—)	6 (+)	6 (—)	Mean values ± S.D. (+)	Mean values ± S.D. (—)
Dose	10	5	10	5	—	—
C_o	82	39	80	43	—	—
τ_{el}	12.9	31.7	8.6	30.2	13.0 ± 3.2*	29.0 ± 8.8*
V_f	122	128	125	116	127 ± 12	122 ± 12
k_{el}	9.4	4.0	14.5	3.8	10.2 ± 2.4*	4.6 ± 2.0*

stantial differences in K_m or K_s values were found by these groups. It seems likely that the clearance differences are due to differences in rate of metabolic conversion of the antipodes by the mixed function oxygenase system.

Proteinbinding

The binding of (+)-, (—)- and (±)-hexobarbital to 3% serum albumin was investigated by equilibrium dialysis at pH 7.4 and 37° C. The binding was determined at three barbiturate concentrations which were in the range of those encountered in vivo (14.75, 29.5 and 59 μg/ml). The results obtained (Table IX)

TABLE IX. Proteinbinding of (+)-, (—)- and (±)-hexobarbital*.

Conc. / Comp.	0.063 mMol	0.125 mMol	0.250 mMol
(+)	25.4 ± 1.1 (7)	26.0 ± 1.0 (6)	21.4 ± 1.3 (5)
(—)	27.2 ± 1.6 (4)	26.5 ± 1.1 (4)	22.5 ± 0.3 (4)
(±)	28.5 ± 2.9 (5)	24.5 ± 1.2 (6)	20.6 ± 0.8 (4)

* Mean values ± S.E.; 3% BSA; phosphate buffer pH 7.4; 37° C. Number of experiments in parentheses. The differences in protein binding of the compounds at the same concentration were not statistically significant (Student's t-test, $P > 0.05$).

show that, although there is substantial binding at all three concentrations, at no concentration a statistically significant difference in binding between the antipodes exists ($P > 0.05$). It can be concluded that there is no stereospecific interaction between albumin and the hexobarbital antipodes.

DISCUSSION

In the present experiments it was shown that the same rat slept much longer after receiving (+)-hexobarbital than after receiving the same dose of (—)-hexobarbital. There are at least four possible explanations for this observation:

1. (+)-hexobarbital is eliminated from the brain at a slower rate;
2. (+)-hexobarbital is metabolized into an active metabolite to a greater extent;

3. there exists a stereoselective distribution process, such that more (+)-hexobarbital can pass into the brain or throughout the brain to the active sites;
4. (+)-hexobarbital is more potent at the active site.

Our detailed pharmacokinetic study probably does provide information which allows a distinction between these four possibilities.

1. The rate of metabolism is the determining factor in the elimination of hexobarbital from blood and because equilibrium between plasma and brain is achieved very rapidly after i.v. injection (Richards and Taylor, 1956), this will also be true for the rate of elimination from the brain as shown in mice by Noordhoek (1969). It was found that (+)-hexobarbital disappears two to three times faster from the blood than (—)-hexobarbital, while the contrary could have explained the longer sleeping time.

2. Norhexobarbital (N-demethylated hexobarbital) is the only known metabolite of (±)-hexobarbital that has anaesthetic properties (Bush et al., 1953). Although our gaschromatographic assay was sufficiently sensitive and selective for the determination of norhexobarbital in blood, (the pure compound was synthesized for reference purposes), only traces could be detected after administration of the two antipodes. These results are in contrast with those of Rummel et al. (1967), who found substantial amounts of norhexobarbital in serum after administration of (+)-and (—)-hexobarbital to female rats. These investigators measured norhexobarbital by UV-spectrophotometry after TLC-separation. We checked this procedure, after i.p. administration of 100 mg/kg (±)-hexobarbital to female Wistar rats (150 g) and found that the spot on TLC of a serum sample extract with the same R_f value as authentic norhexobarbital, certainly was not the demethylated product. This was verified with the aid of combined gas chromatography-mass spectrometry. So it seems very unlikely that the norhexobarbital does contribute to the duration of anaesthetic activity of one of the hexobarbital antipodes.

3. Distribution of most body foreign substances from the blood into the brain occurs by passive diffusion and is determined by lipophility, pKa-value and plasma protein binding (Goldstein et al., 1969). As optical antipodes have the same physico-chemical properties except optical rotation, their intrinsic ability to pass the blood-brain barrier by passive diffusion must be considered to be equal. Only the free fraction in the plasma contributes to penetration into brain and other tissues, so a higher free concentration would result in a higher brain level. For the antipodes of N-methyl - 5-cyclohexenyl - 5-ethylbarbital and of N-methyl - 5-phenyl - 5-ethylbarbital, both structurally closely related to hexobarbital, differences in in vitro albumin binding at low protein

concentration have been shown by Büch et al. (1970). At 4% albumin concentration however the differences became less pronounced. We studied the binding of the hexobarbital antipodes to 3% serum albumin in order to ascertain whether differences in binding did exist. The results show that binding in vitro is equal for the two compounds at each concentration investigated. Thus it is very unlikely that a difference in protein binding is a contributing factor to stereoselective brain penetration of the hexobarbital enantiomers. Great differences in distribution behaviour between the antipodes would also have been reflected in different volumes of distribution (V_1 and V_f) or in the distribution rate constants (r_{12} and r_{21}). Although there is quite some variation in these parameters (Table VIII), significant differences between the two compounds do not exist in this respect.

So the pharmacokinetic results provide no reasons to assume that the hexobarbital antipodes distribute in a stereoselective way in rats. However, Rummel et al. (1967) found that (+)-hexobarbital achieved a higher brain/blood concentration ratio than (—)-hexobarbital between 1 and 7 min after i.v. administration. At 7 min the difference is only small and as one might assume that equilibrium between plasma and tissues has been reached by this time, it does not explain the great difference in sleeping time between the antipodes. Obviously, a stereoselective distribution throughout the brain cannot be excluded.

4. Wahlström et al. (1970) already showed that the hexobarbital antipodes had unequal anaesthetic potency, despite equal brain concentration. As equilibrium between plasma and brain concentration of hexobarbital is very rapid (Richards and Taylor, 1956), the plasma or blood concentration is a good reflection of brain concentration. Therefore, the blood concentration at the moment of awakening, which can be considered as the minimum effective level to cause anaesthesia, is a measure for central depressant potency. This level is approximately three to four times lower for (+)-hexobarbital than for (—)-hexobarbital. Even if one assumes a somewhat higher brain/blood ratio for (+)-hexobarbital, it still can be concluded that at least a 2- to 3-fold higher blood concentration of (—)-hexobarbital is required to produce anaesthesia in rats.

The fact that the latency time was less than 10 sec after i.v. administration of the two compounds can be explained by the high lipophility of hexobarbital, which does allow rapid brain penetration, and by the high blood flow to the brain. There is an immediate building up of high concentration in the brain after i.v. injection, which in most cases far exceeds the minimum effective level. This in contrast to i.p. administration, when first diffusion of the compounds to the bloodstream must take place and now it takes some time before the minimum effective level has been reached. As this is higher for (—)-hexobarbital it seems rational that more time for this isomer is required. It explains

the difference in latency after i.p. administration reported by Rummel et al. (1967) and Furner, Mc Carthy et al. (1969). Our finding that there is quite some variation in minimum effective level for (—)-hexobarbital can be explained by the fact that awakening occurs mostly already during the distribution phase, when a rapid change of concentration occurs. The equilibrium state concentration is below the minimum effective level, so that a more accurate value cannot be obtained from these experiments.

In conclusion it can be stated that the results strongly suggest that a stereospecific mechanism at the active sites in the CNS is involved in hexobarbital anaesthesia. It certainly could explain the differences noted in sleeping time after administration of the antipodes to rats.

APPENDIX

The equations for the two-compartment open model are valid if i.v. injection is instantaneous. In fact injection lasted 10 sec = 0.17 min and therefore the experimental coefficients $A_1{}^*$ and $A_2{}^*$ have to be corrected. Injection is looked upon as a linear i.v. infusion and it can be derived that:

$$A_1 = \frac{A_1{}^* \cdot 0.17}{\tau_1 (1 - e^{-0.17/\tau_1})}$$

$$A_2 = \frac{A_2{}^* \cdot 0.17}{\tau_2 (1 - e^{-0.17/\tau_2})}$$

The other pharmacokinetic parameters have been calculated according to the equations given in Chapter 1, Section I.

SUMMARY

Pharmacokinetics in man

The pharmacokinetics of (+)-, (—)- and (±)-hexobarbital were studied in five healthy volunteers after oral administration. Each volunteer received the three compounds (400 mg) with an interval of about 2 weeks. Hexobarbital plasma concentrations were determined at regular times after drug administration.

The elimination half-life of (—)-hexobarbital was approximately 3.2 times shorter than that of (+)-hexobarbital (1.4 and 4.6 h respectively). From the areas under the plasma concentration curves it was estimated that about 18% of the administered dose of the (—)-isomer was metabolized during first passage through the liver ("first-pass effect"). This is due to its high metabolic clearance. The elimination phase

of the racemic mixture (average half-life 4.0 h) should not give, in principle, a straight line. However, it was discussed that after oral administration the contribution of the (—)-isomer to the pure elimination phase of the racemate was almost negligible. An additional experiment revealed that after rapid i.v. infusion of the racemic mixture it may be possible to distinguish between the different elimination rates of the two enantiomers. Hexobarbital was hardly excreted unchanged into the urine after administration of the three compounds, whereas (—)-hexobarbital was metabolized to 3-keto-hexobarbital to a greater extent than (+)-hexobarbital.

The volunteers experienced severe drowsiness after (+)-hexobarbital administration. It seems likely that this isomer has a greater central depressant activity than the (—)-isomer, although the differences in disappearance rate also may explain the differences in observed activity.

Pharmacokinetics in rats and in the isolated perfused rat liver

The pharmacokinetics of (+)- and (—)-hexobarbital were studied in the same intact rat after i.v. administration, as well as in the same isolated perfused rat liver. The blood half-life of (+)-hexobarbital was 2 - 3 times shorter than that of (—)-hexobarbital, both in vivo and in vitro. No differences in apparent volume of distribution were observed, but the metabolic clearance constant of (+)-hexobarbital was 2 - 3 times greater. Clearance values in vivo were found to be equal to those in vitro for the same compound. No differences in binding to 3% albumin were observed at 3 physiological concentrations, determined by equilibrium dialysis. The animals slept much longer after injection of (+)-hexobarbital and the blood concentration at the moment of awakening was approximately 4 times lower than after injection of (—)-hexobarbital. It is suggested that a difference in anaesthetic potency between the hexobarbital enantiomers at the CNS level is likely to exist.

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METHOHEXITAL SODIUM INTRAVENOUS AND ORAL ADMINISTRATION

INTRODUCTION

Methohexital sodium (BRIETAL) was introduced in 1959 as an intravenous anaesthetic agent. The drug has been investigated, for this purpose, in several clinical trials which have been reviewed recently by Whitwam (1972). Methohexital was found to be one of the most potent intravenous induction agents and it is approximately three times as potent as thiopental (Thomas, 1967; Clarke et al., 1968). Complete recovery after methohexital sodium administration from anaesthesia has been observed and this is significantly more rapid than for thiopental sodium (Egbert et al., 1959; Elliott et al., 1962; Barry et al., 1967). This is beneficial to the patient and also the hospitalisation during the day cases can be safely shortened when using methohexital (Barlow and Gottlich, 1962; Whitwam, 1972).

Methohexital sodium has been included in the present investigations, because of the rapid and complete recovery that has been reported after i.v. administration. Also, after oral administration (Bush et al., 1966) recovery was found to be complete within a few hours. This may indicate that the drug is rapidly inactivated in the body, which is an advantage when the drug is used in the treatment of insomnia. Many patients have difficulty in getting to sleep, but experience little difficulty in remaining in that state once achieved. In such cases satisfactory therapy may be achieved by the use of a short-acting barbiturate (Way and Trevor, 1971).

The metabolism and excretion of the drug have been studied in both rat and dog by Welles et al. (1963) and in man by Brand et al. (1963). In the latter study, however exceptionally high doses of at least 1.2 g were given. Furthermore, Sunshine et al. (1966) measured blood levels after clinical doses (1.5 - 2 mg/kg) of methohexital, by gas chromatography. The sensitivity of their assay was such, that it was not possible to measure concentrations for more than 10 min after drug administration. Bush et al. (1966) included methohexital

sodium in a study concerning ultra short-acting barbiturates as oral hypnotic agents in man. Blood levels up to 3 or 4 h after drug administration (7 mg/kg) were determined and it appeared that both absorption and distribution or elimination were relatively rapid. Due to the limited amount of data, however, the accurate determination of a half-life from these experiments was not possible. Improvements in the sensitivity of the assay for methohexital (Chapter 4, Section II) now make it feasible to determine the pharmacokinetics after administration of therapeutic doses to man. Firstly, the drug was administered intravenously in order to establish an appropriate pharmacokinetic model and also to accurately calculate parameters. Administration occurred by slow zero-order i.v. infusion in order not to discomfort the volunteers and to avoid systemic reactions. Secondly, methohexital sodium was administered orally and the rate of absorption and elimination was estimated.

METHODS

Intravenous administration

Methohexital sodium (BRIETAL sodium) for i.v. injection was obtained from LILLY, USA. Solutions for i.v. infusion were prepared, immediately before use, by dissolving the sterile contents of a sealed container in sterile physiological salt solution (10 mg/ml).

Four healthy male volunteers, ranging in age from 23 - 27 years and in bodyweight from 55 - 81 kg, participated in the study of i.v. administration. They had received no regular medication during the 4 weeks preceding initiation of the experiments. After an overnight fast, the volunteers received methohexital intravenously by a 60 min zero-order infusion. The doses applied were 3 mg/kg of the sodium salt. The volunteers were resting supine during infusion and for 3 h after infusion. Blood samples were taken from a forearm vein at the following times during infusion: 10, 20, 30, 40, 50 and 60 min. After infusion blood samples were withdrawn usually after 5, 10, 15, 25, 35, 45, 55, 75, 95 min and after 2, 3, 4, 5 and 6 or 7 h.

Oral administration

Methohexital sodium for i.v. injection (BRIETAL sodium) was also used for the oral experiments. Hard gelatine capsules were filled with the appropriate amount of drug. Four healthy male volunteers (two of them had received methohexital by i.v. infusion), ranging in age from 21 - 27 years and in body-

weight from 74 - 81 kg participated in the study of oral administration. After an overnight fast, the volunteers received methohexital sodium (3 mg/kg) together with 150 ml water. They were requested to lie down for at least 2 h after drug administration. Blood samples were taken at the following times: 10, 20, 30, 45, 60 min and 1.5, 2, 2.5, 3, 4, 5 and 6 h after drug administration.

Methohexital plasma concentrations were determined by gas chromatography as described in Chapter 4, Section II.

RESULTS AND DISCUSSION

Intravenous infusion

During i.v. infusion of methohexital sodium a rapid rise in the methohexital plasma concentration occurred until the zero-order infusion was terminated. After this event the plasma concentration-time curve exhibited at least two phases; an early phase with relatively steep slope and a latter phase with a more gradual slope (Fig. 1). This suggests that the kinetics of methohexital after i.v. administration fit the two-compartment open model. The entire curve may be represented mathematically as a sum of two exponentials, as has been outlined in detail for hexobarbital (Chapter 1, Section III). The methohexital plasma concentrations were fitted according to the equations which are valid during and after infusion, by using the FARMFIT computer program. The initial graphical estimates of τ_1 and τ_2 were used as a start guess. A relative error of 5% in the concentrations was taken into account, since this was the standard deviation estimated in the assay procedure (Chapter 4, Section II). An example of a fitted and plotted curve is given in Fig. 1 and it illustrates the agreement between the plasma levels and the two-compartment model. The consistency also follows from the relatively small standard errors calculated for the fitted parameters (Table I). In contrast with hexobarbital, the concentrations for methohexital during the infusion period were included in the fitting procedure. It appeared that by doing this, the fit was improved as judged by the smaller standard errors in the fitted parameters. Since in most cases the concentrations 5 or 6 h after infusion were higher than expected on the basis of the two-compartment kinetics, the possibility of the existence of a third compartment was considered. The fitting procedure was now accomplished by the assumption of a three-exponential equation underlying the concentration course during and after infusion (Eq. 5 and 6, Chapter 1, Section III). In Fig. 2 an example of such a fitted curve is given for the same experimental data of Fig. 1. Although a good fit of the data to the curve is apparent, the standard

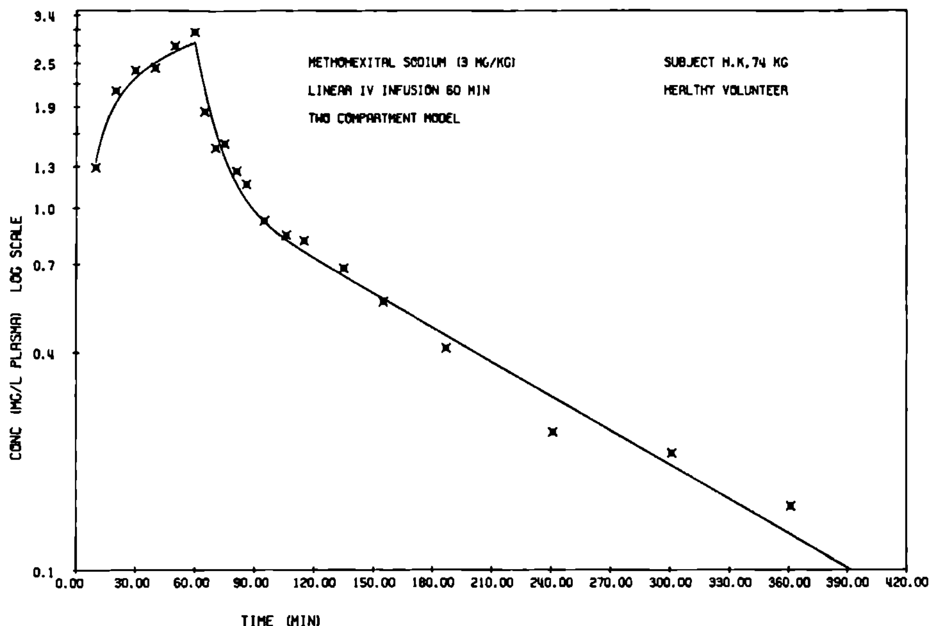

FIG. 1. Methohexital plasma concentration curve (semi-logarithmic scale) during and after a 60 min zero-order i.v. infusion of methohexital sodium to a healthy volunteer. The concentrations were fitted according to Eq. 5 and 6, Chapter 1, Section III, assuming that a two-compartment model is valid for methohexital i.v. kinetics. See Table I for the fitted parameters, including standard errors. The reproduction was obtained from a direct computer plot.

errors in the fitted parameters are much higher than in the case of a two-compartment model. This is due to the fact that six parameters had to be fitted with the same amount of experimental data. Unfortunately the sensitivity of the assay did not allow measurements of the plasma concentrations for longer periods of time. For comparative purposes the fitted parameters of the three-compartment model are given in Table II. A reasonable three-compartment fit was obtained only for two of the volunteers (H.K. and R.C.). A three-compartment fit of the data was not possible for the other two volunteers. The present experiments do not indicate that the use of the three-compartment model is valid for methohexital kinetics. Further experiments, with higher doses, or repeated administration are needed in order to follow plasma concentrations for longer periods of time. It seems likely that methohexital will be similar to thiopental and hexobarbital in its pattern of distribution immediately after i.v.

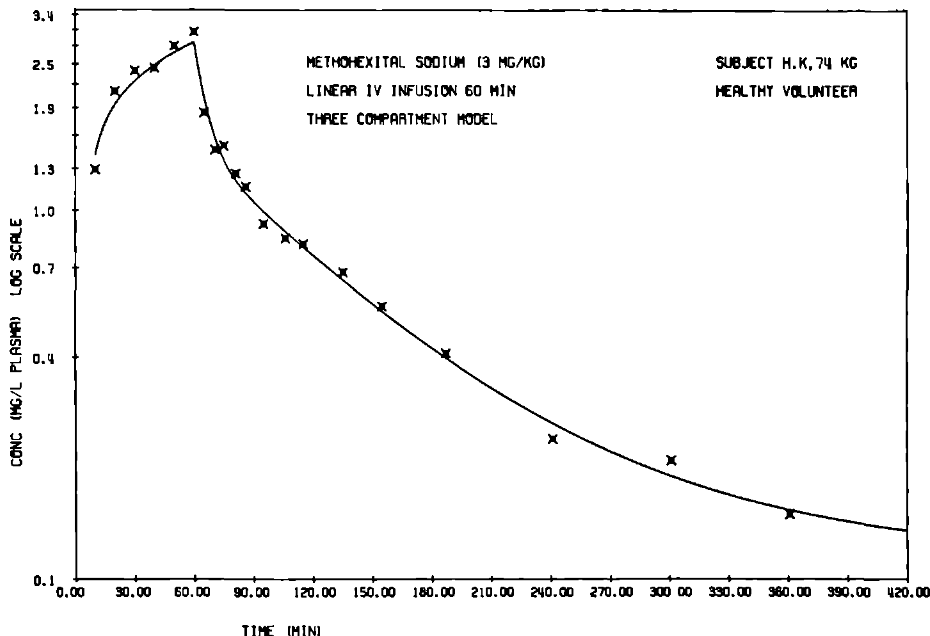

FIG. 2. Methohexital plasma concentration curve (semi-logarithmic scale) based upon the same experimental data as in Fig. 1. In this instance, the concentrations were fitted according to Eq. 5 and 6, Chapter 1, Section III, assuming that a three-compartment model is valid for methohexital i.v. kinetics. See Table II for the fitted parameters, including standard errors. The reproduction was obtained from a direct computer plot.

injection. Rapid recovery of consciousness, after a single i.v. dose of each of these drugs, is caused largely by a redistribution of the drugs from the highly perfused viscera, including the central nervous system, into lean body mass (Price et al., 1960). However, methohexital as well as hexobarbital, are less extensively localized in fat tissue than thiopental (Brand et al., 1963). If fat uptake plays a minor role in methohexital elimination, then there is no important physiological reason to suppose that a three-compartment model is required to explain methohexital kinetics. It has been shown for thiopental that it takes a few hours before equilibrium is reached between the concentration in blood and in adipose tissue (Price et al., 1960). Probably the metabolism of methohexital is so rapid that the poorly perfused fat tissue does not experience substantial drug uptake. Only for very high doses would it become a factor to be considered. The plasma levels obtained by Brand et al. (1963),

TABLE I. Fitted and calculated pharmacokinetic parameters of methohexital, according to a two-compartment open model, after i.v. infusion of methohexital sodium to four healthy volunteers (standard errors in parentheses).

Name	H.K.		R.C.		R.H.		P.A.*	Mean
Age (yr)	23		25		27		24	24.8
Body-weight (kg)	74		55		81		64	68.5
Dose (mg free acid)	204		152		224		177	189.3
Dose/kg (mg)	2.76		2.76		2.76		2.76	2.76
Inf. time (min)	60		60		60		60	60
τ_1 (min)	9.7	(14.7%)	9.8	(17.2%)	10.1	(25.4%)	7.2	9.2
τ_2 (min)	138	(6.4%)	181	(12.8%)	141	(6.5%)	101	140
$t_{1/2}$ (min)	95		125		97		70	97
A_1 (mg/l)	10.7	(12.8%)	9.87	(16.8%)	6.67	(34.9%)	7.50	8.69
A_2 (mg/l)	1.38	(8.4%)	0.74	(13.3%)	1.11	(8.5%)	1.30	1.13
V_1 (l)	16.8		14.3		28.7		20.1	20.0
V_1/kg (l)	0.23		0.26		0.35		0.31	0.29
V_f (l)	64.1		71.7		101.4		70.4	76.9
V_f/kg (l)	0.87		1.30		1.25		1.10	1.13
k_{el} (ml/min)	692		657		999		955	829
k_{el}/kg (ml/min)	9.35		11.95		12.33		14.93	12.14
k_{12} (ml/min)	858		702		1465		1455	1120
r_{12} (1/min)	0.051		0.049		0.051		0.072	0.056
r_{21} (1/min)	0.018		0.012		0.020		0.029	0.020
AUC (mg.min/l)	295		232		224		185	234
AUC/kg (mg.min/l)	3.99		4.22		2.77		2.89	3.47

* Experimental parameters graphically determined by hand.

TABLE II. Fitted and calculated pharmacokinetic parameters of methohexital, according to a three-compartment open model, after i.v. infusion (standard errors in parentheses).

Name	H.K.		R.C.	
τ_1 (min)	3.8	(34.7%)	3.4	(29.2%)
τ_2 (min)	63.4	(32.1%)	48.0	(16.9%)
τ_3 (min)	415	(128%)	557	(49.9%)
A_1 (mg/l)	22.7	(38.6%)	19.6	(24.9%)
A_2 (mg/l)	2.02	(11.0%)	1.68	(12.8%)
A_3 (mg/l)	0.31	(111%)	0.25	(29.8%)
k_{el} (ml/min)	595		530	
V_f (l)	108		150	

after i.v. infusion of 1.2 to 1.8 g methohexital to four volunteers, indicate that there may be a slower phase with these high doses, corresponding to a half-life of 4 - 5 h. However, in these experiments plasma levels were not followed long enough to draw definite conclusions. Further, it should be considered that methohexital is used in practice as a racemic mixture (Chapter 4, Section II). Similar to hexobarbital, the possibility exists that the kinetics of methohexital are more complex due to differences in elimination rate of the two enantiomers.

In Table I the pharmacokinetic parameters of methohexital are given, according to the two-compartment open model. The apparent volume of distribution is in the same order of magnitude as found for hexobarbital. The elimination half-life of the drug is very short, which is mainly due to the high metabolic clearance. Obviously, the clearance is smaller when calculated according to a three-compartment model, whereas the apparent volume of distribution is much larger (Table II). Sunshine et al. (1963) found only small amounts of methohexital in urine and bile of man; less than 1% of the administered dose was excreted as unchanged methohexital. Welles et al. (1963) studied the metabolism and excretion of methohexital in the rat and the dog. They found no unchanged drug in the urine, but by using paper chromatography they found evidence for the existence of several metabolites in urine. The most abundant of these metabolites was identified as 1-methyl-5-allyl-5-(1-methyl-4-hydroxy-2-pentynyl)-barbituric acid which is formed by hydroxylation of the penultimate carbon of the pentynyl side chain. Demethylation of the N-methyl group did not occur to a great extent. An interesting observation has been that faecal excretion of metabolites in rats amounted to 82% of the administered

dose, whereas in the dog this value was 30%. In man no data on metabolic pathways or excretion routes in quantitative terms are available.

It is highly probable that the rapid and complete recovery after methohexital administration, after regaining consciousness, is due to the rapid inactivation of the drug by metabolism. The present findings substantiate earlier speculations in this direction and in conclusion it may be stated that this is of great benefit for the use of methohexital in i.v. anaesthesia for operations of short duration. It should be mentioned that in the present experiments the volunteers experienced drowsiness and were inclined to sleep generally fifteen minutes after the commencement of infusion. Throughout the infusion period they could however, easily be awakened and after termination of infusion recovery was complete apparently within one hour, as judged subjectively.

Oral administration

Methohexital sodium, after oral administration in a hard gelatine capsule, gave results indicative of rapid absorption. The peak level times and the maximum concentrations reached, are given in Table III. A rapid absorption was expected, since salts of barbiturates are, in general, rapidly absorbed, due to their high dissolution rate in the gastrointestinal tract (compare hexobarbital sodium, Chapter 1, Section III and heptabarbital sodium, Chapter 5, Section III). In all cases the volunteers reported initial feelings of euphoria, followed by symptoms of sleepiness for about half an hour, including the times of the

TABLE III. Pharmacokinetic parameters of methohexital after oral administration of methohexital sodium to four healthy volunteers.

Name	S.R.	H.R.	R.H.	H.K.	Mean
Age (yr)	24	25	27	23	24.8
Body-weight (kg)	78	76	81	74	77.3
Dose (mg free acid)	217	210	224	204	214
Dose/kg (mg)	2.76	2.76	2.76	2.76	2.76
t_{max} (min)	30	60	30	20	35.0
C_{max} (mg/l)	0.77	0.42	0.98	1.20	0.84
$t_{1/2}$ (min)	43	46	62	55	51.5
AUC (mg.min/l)	51	35	98	100	71
AUC/kg (mg.min/l)	0.65	0.46	1.21	1.35	0.92

peak plasma concentration. Also, subject H.R. with the lowest peak concentration, experienced drowsiness for a short time. Recovery in all cases was apparently complete within one hour after the peak level time. A dose of 3 mg/kg should be sufficient to induce sleep at night quite rapidly.

The elimination phase after oral methohexital administration could in all cases be described by a single first-order process (Fig. 3), however, the half-life

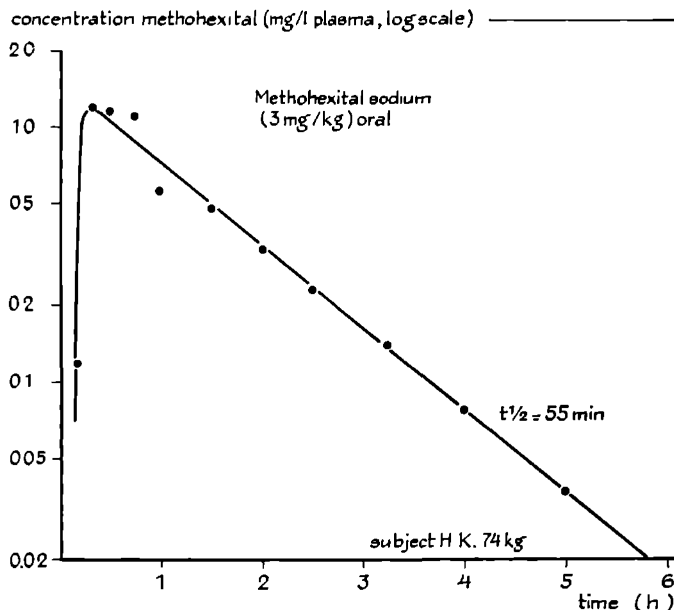

FIG. 3. Methohexital plasma concentration curve (semi-logarithmic scale) following oral administration of methohexital sodium (3 mg/kg) in a hard gelatine capsule to a healthy volunteer.

was shorter than after i.v. infusion. The reason for this finding is not clear, although a possibility exists that a combination of pure elimination and distribution of the drug into peripheral tissues, is actually measured. The curve could not be followed long enough to see whether a distinct second phase with a longer half-life existed. The high metabolic clearance after i.v. administration suggests that a "first-pass effect" may influence the bioavailability of methohexital after oral administration to a considerable extent (Rowland, 1972). The average ratio of the areas under the plasma concentration curves is a measure of the bioavailability. In Table I these values are given for i.v. administration, obtained by dividing dose by the clearance constant (AUC =

D/k_{el}). The AUC's of the oral experiments were determined by weighing the areas up to 5 or 6 h and correcting for the remaining area by adding the product of the last concentration measured and the elimination time constant, to the experimental area (Dost, 1968). Results are given in Table II and it appears that the average AUC/kg of body-weight is only 26% of the i.v. value (0.92 versus 3.47 mg.min/l). For the volunteers who participated in both experiments (H.K. and R.H.) this value was 34% and 44% respectively. When assuming that pharmaceutical factors are not involved in lowering bioavailability after oral administration, these results would indicate that there is a considerable "first-pass effect" of methohexital after oral administration. It is possible that the effect is overestimated due to the short half-life after oral administration, since this may indicate that the clearance was higher than after i.v. infusion. In that case the oral AUC-values are not comparable with the i.v. values, since it is the product of AUC and clearance which have to be compared. Thus bioavailability may be slightly higher than the determined average value of 26%. Nevertheless the present findings are in agreement with the theory that a substantial amount of a drug, with a high clearance when administered orally, is metabolized during first passage through the liver.

CONCLUSIONS

Methohexital is rapidly metabolized in man, which is highly favourable when anaesthesia is required for operations of short duration. The rapid and complete recovery, which is reported in the literature, after i.v. methohexital anaesthesia is in agreement with the rapid inactivation of the drug.

After oral administration, methohexital sodium is rapidly absorbed, and its half-life appears to be even shorter than after i.v. administration. Bioavailability is low, due to a considerable "first-pass effect". Methohexital sodium may be used orally for the rapid induction of sleep, although further experiments would be required in order to ascertain the optimal dose and the pharmaceutical formulation in which the drug should be incorporated.

SUMMARY

Methohexital kinetics were studied in man after i.v. and oral administration of methohexital sodium. For the i.v. experiments the drug was administered by a 60 min zero-order infusion (3 mg/kg) to four healthy volunteers. During and after infusion the plasma concentrations were determined at regular intervals. The concentrations were fitted according to the equation that is valid for both a two-compartment open

model (Fig. 1) and a three-compartment open model (Fig. 2). In the latter case a satisfactory fit could be obtained for only two of the volunteers. It was discussed that two-compartment kinetics may be more valid for methohexital during and after i.v. infusion. Elimination of methohexital in the volunteers was extremely rapid; plasma half-lives of 70 - 125 min were estimated. This is due to the high metabolic clearance of the compound, which varied from 657 to 999 ml/min. The average volume of distribution was 1.13 l/kg. It was concluded that the rapid inactivation of the drug in the body leads to a rapid and complete recovery when the drug is used as an intravenous anaesthetic agent for operations of short duration.

After oral administration of methohexital sodium to four volunteers (3 mg/kg), in a hard gelatine capsule, rapid absorption was noted. The elimination half-lives were shorter than after i.v. administration (43 - 62 min). Due to the high metabolic clearance a substantial "first-pass effect" was evident. An average bioavailability of 26% was estimated for methohexital after oral administration. It was concluded that methohexital sodium may be used orally for the rapid induction of sleep.

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VINYLBITAL
ORAL AND RECTAL ADMINISTRATION

INTRODUCTION

Vinylbital is one of the newer barbiturates which was introduced into therapy in 1963. It is used in the treatment of insomnia and also as a sedative agent. In the Netherlands the drug is available in tablet form under the trade name BYKONOX, while in France suppositories also are in use (SUPPOPTANOX). The tablets contain 150 mg vinylbital and the suppositories 200 mg. Several reports have appeared on the effectiveness of the drug in the treatment of insomnia in patients with various diseases and also in healthy people (Zara, 1963; Dumrauf, 1964; Perkhof, 1964; Baptist, 1965; Beck, 1968; Dziuba, 1968; Meyer, 1968). Jovanovic (1967a) extensively studied the effect of vinylbital on the sleeping process in patients with slight to marked disturbances of sleep. He concluded that the drug was able to restore sleep, more or less to normal, and also that side effects, both encephalographic and clinical, were fewer than with other barbiturates. Also, REM sleep was not greatly influenced after a normal dose of 150 or 200 mg. The same author (Jovanovic, 1967b) reported on the neurophysiological aspects of vinylbital action.

Although Hofmann (1971) measured some vinylbital blood levels in man after relatively high doses, no detailed pharmacokinetic information is available. Therefore, it was decided to study the rate of elimination, the rate of absorption and the relative bioavailability, both after oral (BYKONOX) and after rectal (SUPPOPTANOX) administration of vinylbital. Furthermore, plasma levels during chronic vinylbital administration were determined, in order to see if accumulation of the drug in the body would occur and also to ascertain whether the half-life would remain unchanged.

METHODS

The BYKONOX tablets and SUPPOPTANOX suppositories were obtained from BYK-Nederland, Zwanenburg, The Netherlands.

The vinylbital content of the tablets was determined at the Dutch Pharmacist's Laboratory, The Hague, by potentiometric titration with alkali (0.1 N) in nitrogen atmosphere. An average content of 147.5 mg (98.3%) was found, with a variation coefficient of 1%. The suppository base consisted of a mixture of polyethylene glycols. The vinylbital content of the suppositories was determined by gas chromatography, after extraction of the active ingredient by diethyl ether. An average content of 198 mg (99%) was found, with a variation coefficient of 2%.

Six healthy male volunteers, ranging in age from 19 - 25 years and in body weight from 55 - 84 kg, participated in the study.

They had received no regular medication during the 4 weeks preceding initiation of the experiments. After an overnight fast, the subjects were given 150 mg vinylbital as a BYKONOX tablet with 150 ml water, or 200 mg vinylbital as a SUPPOPTANOX suppository. No food, fluid or tobacco was allowed for 3.5 h after drug administration. At first, the subjects were asked to remain in an upright position for 15 min and then to lie for at least 3.5 h. This procedure was undertaken in order to simulate as closely as possible the situation of taking a hypnotic drug and going to bed. Blood samples (5 ml) were taken from a forearm vein, usually at the following times: 20 min, 40 min, 1, 1.5, 2, 3 (4), 5, 7 (8), 9, 12, 24, 30, 34 and 48 h after administration.

Every subject received both preparations with an interval of at least 2 weeks. The sample schedule was the same in the two trials. The plasma samples were stored frozen until the time of analysis. Three subjects collected their urine during 3 - 4 days after the administration of a BYKONOX tablet. Vinylbital concentration in plasma and in urine was determined by gas chromatography, as described in Chapter 5 of Section II.

Two subjects participated in a study of repetitive vinylbital administration. On the first day of the experiment the half-life of the drug was determined and from the second night on they took one BYKONOX tablet with 150 ml water. The next morning, nine hours after drug intake, a blood sample was taken and the vinylbital concentration was determined. On the eighth day vinylbital was given again in the morning, in order to determine the half-life of the drug after repetitive administration. In Fig. 6 the time schedule of drug intake and blood sampling is indicated.

RESULTS

The individual plasma concentration curves during the first 12 h are shown in Fig. 1 (BYKONOX) and in Fig. 2 (SUPPOPTANOX). Absorption, after

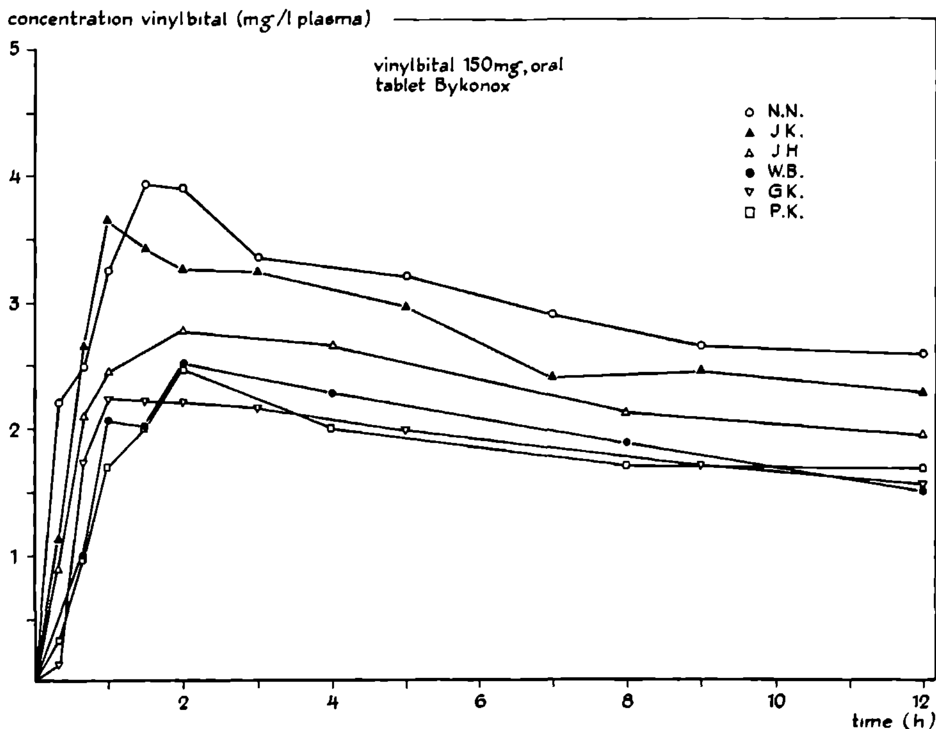

FIG. 1. Plasma concentration curves of vinylbital after oral administration of a BYKONOX tablet to six healthy volunteers.

oral administration, occurs more rapidly than after rectal administration, since the peak levels are reached earlier. This occurs in the first case between 1 and 2 h and in the latter case between 2 and 6 h. Maximum concentrations vary from 2.2 to 3.9 mg/l and from 1.8 to 3.8 mg/l respectively. It is apparent that plasma levels are higher when the body-weight is lower.

CNS depression was experienced by all subjects and they were inclined to sleep after ¾ h - 1½ h when receiving the tablet and somewhat later with the suppositories. It could be deduced, from the plasma concentration curves, that sleepiness consistently occurred when the plasma concentration had reached a value between 2.0 and 2.5 mg/l.

In Fig. 3 the plasma concentration curves of the two preparations, in the same individual, are shown on semi-logarithmic scale. Apparently, in both cases, absorption and elimination are first-order kinetic processes. This is inferred because the descending part of the curves yields a straight line (elimination

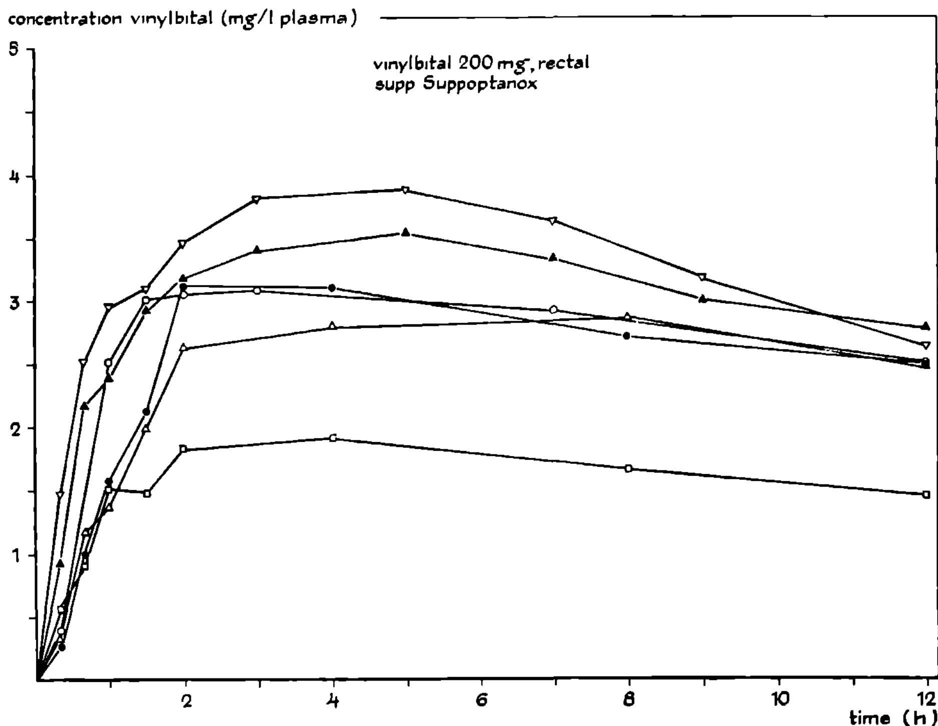

FIG. 2. Plasma concentration curves of vinylbital after rectal administration of a SUPPOPTANOX suppository to the same healthy volunteers as in Fig. 1. (Key the same as in Fig. 1).

phase) and also the absorption phase, obtained with the subtraction method, yields straight lines. The plasma concentration (C_p) can be described by the following equation:

$$C_p = A \cdot (e^{-t/\tau_{el}} - e^{-t/\tau_a})$$

where τ_a and τ_{el} are the time constants of absorption and elimination respectively and A is the "zero-time concentration". The time constants may be deduced easily from the experimental curve, while $\tau_a = 1.44 \times t_{1/2a}$ and $\tau_{el} = 1.44 \times t_{1/2el}$. A is obtained by extrapolating the elimination line and the subtracted absorption line back to zero time. Theoretically these should cross at the same concentration at that time. However, in practice crossing often occurs some time after zero (see Fig. 3). When assuming first order absorption, this means that it lasts some time before absorption starts, which could be due, for example, to a slow

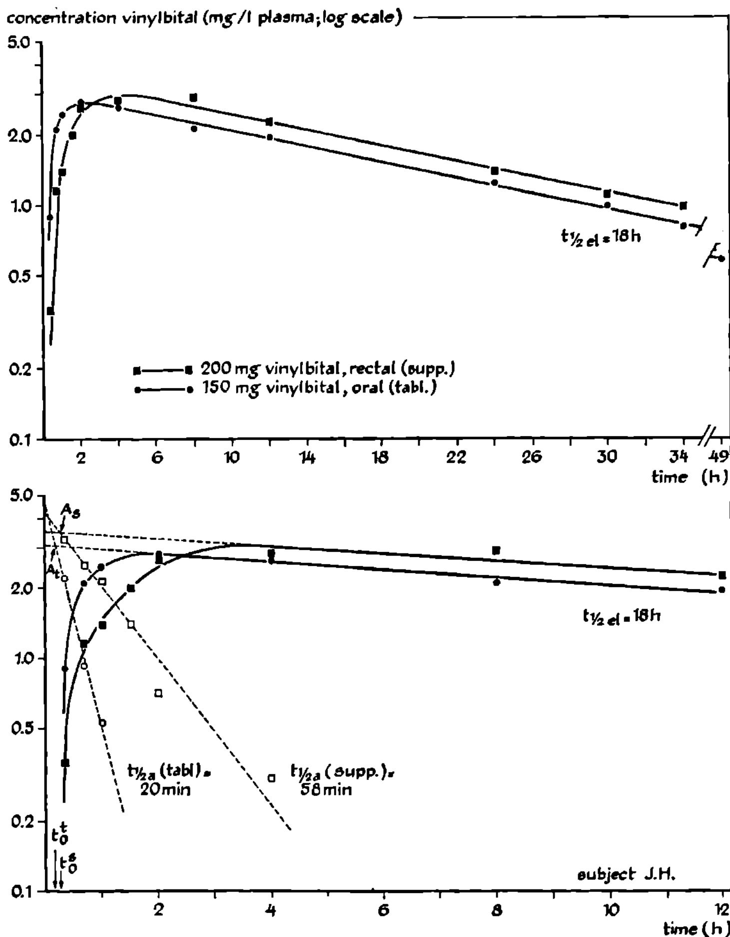

FIG. 3. Plasma concentration curves on semi-logarithmic scale of vinylbital in the same subject after oral and rectal vinylbital administration. *Upper:* time scale up to 49 h; the descending part of the curves represents the elimination phase, which is apparently a first-order process with a half-life ($t_{1/2el}$) of approximately 18 h in both cases. These values were hand calculated while the fitted half-lives were found to be 19.4 h after oral and 17.6 h after rectal administration (Table I). *Lower:* time scale up to 12 h; the dotted lines represent the absorption phase which have been obtained by the subtraction method. Apparently absorption is a first-order process, with half-lives ($t_{1/2a}$) of 20 min and 58 min. A_t is the extrapolated concentration at t_0 (lag time) of the tablet and A_s is the extrapolated concentration at t_0 of the suppository. In Table I the fitted values for $t_{1/2a}$, t_0 and A are given.

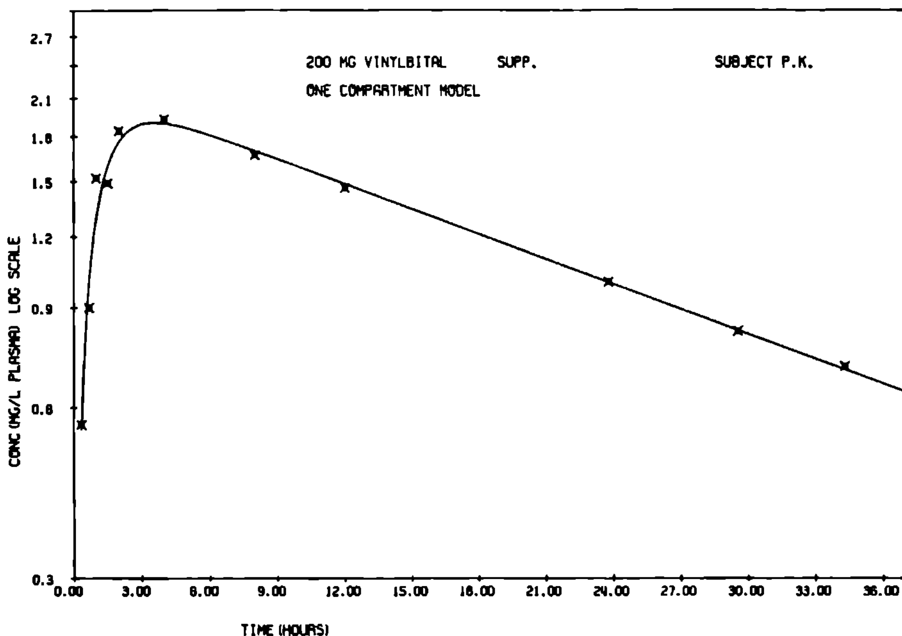

FIG. 4. Example of a fitted and directly computer plotted vinylbital plasma concentration curve. See Table I for the parameter values.

disintegration of the dosage form. This "lag time" (t_0) has to be taken into account in the above equation, where $t = t_{exp}-t_0$. For the extrapolated concentration at t_0 the symbol "A" is used: "zero-time concentration".

All of the individual plasma concentration curves have been fitted according to the above equation (including correction for the lag time) by means of the FARMFIT computer program (fitted parameters: τ_a, τ_{el}, A and t_0). An example of such a fitted and plotted curve is given in Fig. 4. In Table I all of the fitted parameters are given. In most subjects the lag time is shorter with the suppository, indicating that absorption starts earlier from the rectum than from the upper part of the gastrointestinal tract. However, absorption occurred 2 to 4 times faster after oral administration than after rectal administration. Average half-lives of 14.4 min and 38.4 min were obtained, respectively, for the absorption phase. The half-life of elimination of vinylbital varies from 17.6 to 33.5 h, but is reasonably constant within the same subject. Apparently there is no essential difference in elimination rate after oral and after rectal administration, the mean values being 23.5 and 23.8 h.

TABLE I. Fitted pharmacokinetic parameters of vinylbital after oral and rectal administration.

Subject	Body-weight (kg)	Dosage form	Dose* (mg)	Dose/kg (mg)	t_0 (h)	τ_a (h)	$t_{1/2a}$ (h)	τ_{el} (h)	$t_{1/2el}$ (h)	A (mg/l)
N.H.	59	tabl.	150	2.54	0.13	0.22	0.15	31.6	21.9	3.74
		supp.	200	3.39	0.26	0.56	0.38	32.8	22.8	3.47
J.K.	55	tabl.	150	2.73	0.20	0.35	0.24	29.1	20.2	3.52
		supp.	200	3.64	0.10	0.80	0.55	34.7	24.2	3.86
J.H.	75	tabl.	150	2.00	0.20	0.36	0.25	27.9	19.4	3.00
		supp.	200	2.67	0.18	1.37	0.95	25.3	17.6	3.66
W.B.	75	tabl.	150	2.00	0.54	0.21	0.15	37.6	26.0	2.30
		supp.	200	2.67	0.23	1.20	0.83	34.5	23.9	3.58
G.V.	70	tabl.	150	2.14	0.32	0.22	0.15	41.5	28.8	2.24
		supp.	200	2.71	0.01	0.64	0.44	49.8	33.5	3.82
P.K.	84	tabl.	150	1.79	0.23	0.67	0.47	35.1	24.4	2.36
		supp.	200	2.39	0.13	1.01	0.70	29.7	20.6	2.22
mean values		tabl.	150	2.20	0.27	0.34	0.24	33.8	23.5	2.86
		supp.	200	2.90	0.13	0.93	0.64	34.5	23.8	3.44

* These values have not been corrected for the assayed vinylbital content of the dosage forms, since the deviations did not exceed ± 5%.

It can be derived that:

$$A = \frac{D.F}{V_f} \cdot \frac{\tau_{el}}{\tau_{el} - \tau_a}$$

where D is the dose administered, F is the fraction of the drug that reaches the general circulation intact (bioavailability; $0 \leqslant F \leqslant 1$) and V_f is the apparent volume of distribution. With oral or rectal data only, F cannot be estimated, this is only possible by comparison with i.v. administration ($F = 1$). Therefore the quotient V_f/F is calculated, as well as the clearance constant (k_{el}) divided by F ($k_{el}/F = V_f/F.\ 1/\tau_{el}$; Table II). In principle, the relative bioavailability (F_{rel}) of the two preparations in one subject can be easily calculated, by assuming that V_f or k_{el} is constant and taking the tablet as the reference dosage form:

TABLE II. Calculated pharmacokinetic parameters of vinylbital after oral and rectal administration.

Subject	Body-weight (kg)	Dosage form	$\frac{V_f}{F}$ (l)	$(\frac{V_f}{F})$ / kg (l)	$\frac{k_{el}}{F}$ (ml/min)	$(\frac{k_{el}}{F})$ / kg (ml/min)	$F_{rel}\frac{(supp)}{(tabl)}$ x 100%[1] $\frac{(V_f/F)_{tabl}}{(V_f/F)_{supp}}$ (%)	$\frac{(k_{el}/F)_{tabl}}{(k_{el}/F)_{supp}}$ (%)
N.H.	59	tabl.	40.0	0.68	21.0	0.36		
		supp.	58.5		29.7		69	71
J.K.	55	tabl.	43.2	0.79	24.7	0.41		
		supp.	53.0		25.3		82	98
J.H.	75	tabl.	50.6	0.67	30.2	0.40		
		supp.	57.8		38.2		88	79
W.B.	75	tabl.	65.3		29.2			
		supp.	57.9	0.77	28.1	0.37	113	104
G.V.	70	tabl.	67.3		27.3			
		supp.	53.0	0.76	17.7	0.25	127	154
P.K.	84	tabl.	64.9	0.77	30.8	0.37		
		supp.	93.2		52.3		70	59
mean values		tabl.	55.3		27.2			
		supp.	62.2		31.9		92	94
mean of lowest values			51.7	0.74	25.1	0.36		

[1]) The relative bioavailability (F_{rel}) of the suppository has been calculated in two ways, which yield different results. For explanation see text.

$$F_{rel} = \frac{F_{supp}}{F_{tabl}} = \frac{V_f \cdot F_{supp}}{V_f \cdot F_{tabl}} = \frac{(V_f/F)_{tabl}}{(V_f/F)_{supp}} \text{ (x 100\%)}$$

or

$$F_{rel} = \frac{(k_{el}/F)_{tabl}}{(k_{el}/F)_{supp}} \text{ (x 100\%)}$$

The relative bioavailability has been calculated in both ways and it follows from the results in Table II, in most subjects, there are substantial differences between the values obtained. This can be explained by the fact that the time constant of elimination τ_{el} in the same individual is not identical during the

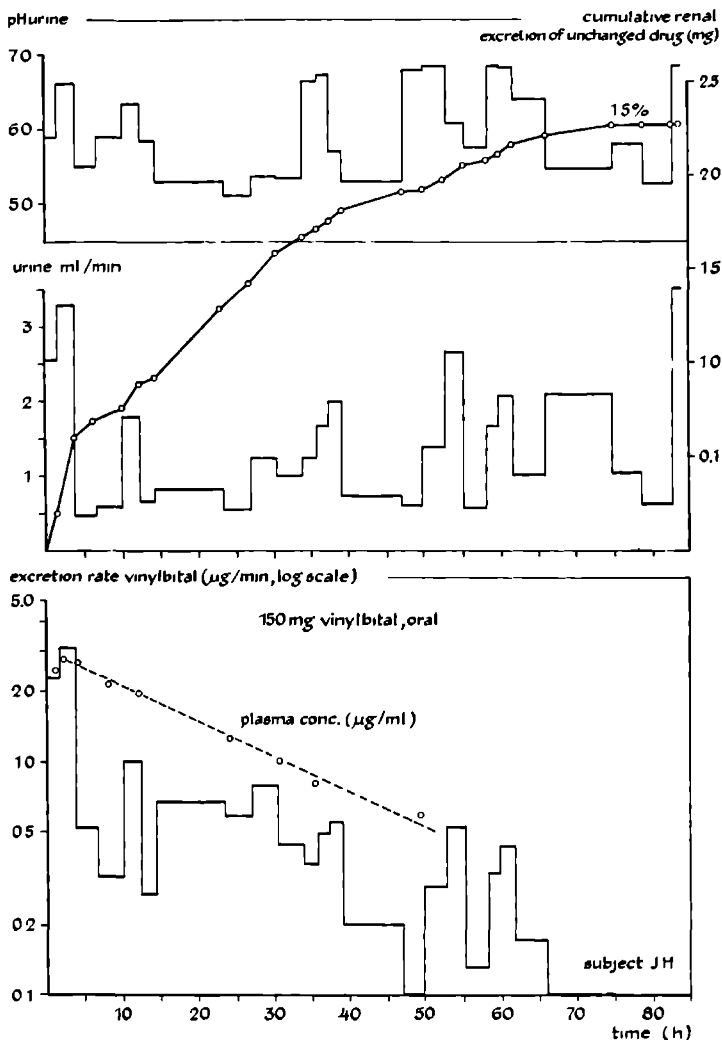

FIG. 5. Renal vinylbital excretion rate, urine pH, urine production and cumulative renal excretion of unchanged drug after oral administration of 150 mg vinylbital. Parallelism can be observed between the plasma concentration curve and the average renal excretion rate curve.

two trials. When single compartment kinetics are valid this must be due either to a change in apparent volume of distribution, to a change in total body clearance or to a simultaneous change in both. Since it is impossible to differen-

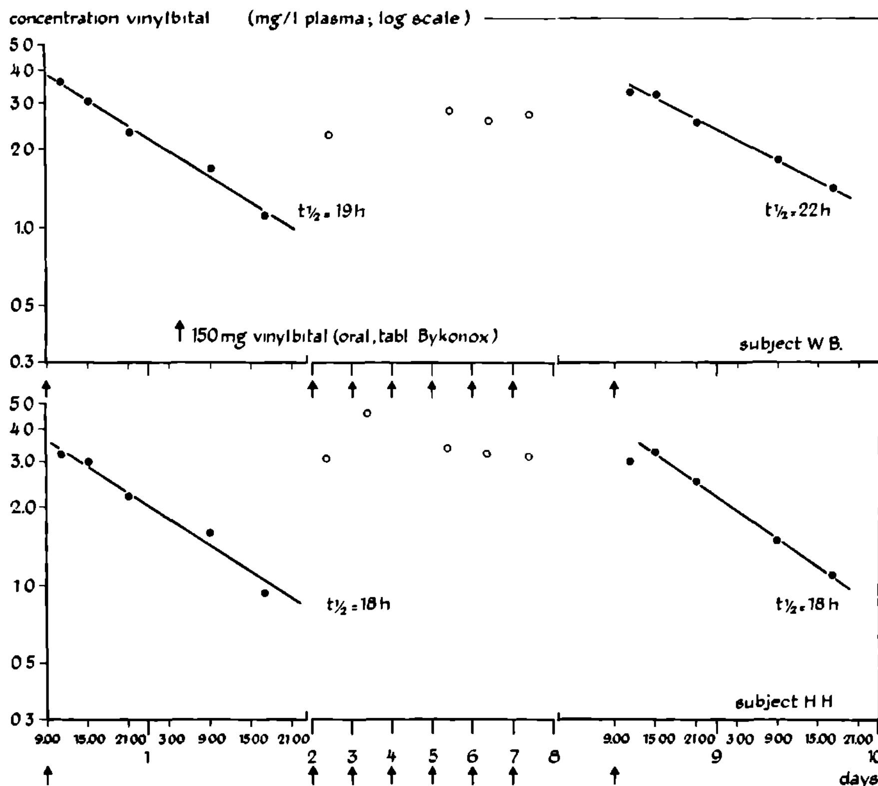

FIG. 6. Half-lives and plasma concentrations of vinylbital before, during and after repetitive vinylbital administration to two healthy volunteers. During the first and second day of the experiment the half-life of the drug was determined. From the second night on a BYKONOX tablet was taken and the next morning (9 h later) the plasma concentration was measured. During day 8 and day 9 the half-life of the drug was again determined.

tiate between these possibilities, one cannot predict which of the two methods of relative bioavailability calculation is preferable. Therefore, the values in Table II should be looked upon as the limits of a range in which the real F_{rel} might be found. In any case, bioavailability of vinylbital from the suppository in four subjects is lower than from the tablet, while the reverse seems true in the two subjects. However, when comparing plasma concentrations, after tablet administration of the latter to the other ones, this observation seems to be due to a low bioavailability of the oral preparation rather than to a high bioavaila-

bility of the suppository. So it seems justified to conclude that the suppository is less capable of delivering vinylbital to the body than the tablet. The difference in dosage (150 mg oral compared to 200 mg rectal), can be considered as a correction for the loss of drug when rectally administered. On the average, the plasma levels do not differ substantially when BYKONOX or SUPPOPTANOX is given, apart from the concentrations during the absorption phase.

Vinylbital appears to be excreted unchanged in the urine to a very small extent. For the three subjects, who collected their urine after the administration of a BYKONOX tablet during 3 - 4 days, cumulative excretion amounted to 1.0, 1.5 and 2.3% of the dose administered. Most probably the remainder is metabolized to more polar metabolites, but no attempt has been made to identify these. The renal excretion rate of vinylbital fluctuates and appears to be dependent on the plasma concentration and also on the urine flow (Fig. 5). A certain parallelism can be observed between the plasma concentration and the average renal excretion rate.

Results of repetitive vinylbital administration are given in Fig. 6. The half-lives in the two subjects, before and after intake of 150 mg vinylbital during a period of six nights, did not differ significantly. Self-induction of the metabolizing enzyme system had not occurred during this (short) period of time. Also, the plasma concentrations at 9 h after drug administration were quite constant. An important accumulation of the drug in the body did not take place, which is in agreement with the fact that the dosage interval (24 h) is greater than one half-life of the drug in these subjects. Nevertheless, a relatively high plasma level after 9 h remains, since by that time only about 20 - 30% of the dose administered has been eliminated.

DISCUSSION

Absorption rate and relative bioavailability

The complete cross-over design in this investigation on vinylbital in man, permits comparison of the pharmacokinetic parameters and also of relative bioavailability in the same subject. This is of advantage when assuming that intrasubject variation is less than intersubject variation with respect to physiological factors. Now differences in absorption rate, lag time or bioavailability of a drug administered in different dosage forms, can mainly be attributed to differences in pharmaceutical formulation or to a different route of administration.

Absorption of vinylbital from the rectum starts as soon as the suppository

has dissolved in the rectum contents, which occurs apparently faster than tablet disintegration in the gastrointestinal tract. This follows from the shorter lag time for the suppository. Also, in the case of oral administration the rate of gastric emptying might be a determining factor, as has been shown for paracetamol (Heading et al., 1973). The dissolution of the active substance in the surrounding fluids is the next step in drug absorption and the rate at which this happens, probably determines the rate of appearance of the drug in blood. This is faster with the tablet and an important reason might be the enormous absorption surface area of the small intestine, together with the greater amount of fluid in the upper part of the gastrointestinal tract, which enhances dissolution of the drug (Benet, 1973). The rectum on the other hand contains a limited amount of fluid, its surface area is relatively small and there occurs almost no convection. Many other factors could influence rectal absorption and these have recently been reviewed by Bevernage and Polderman (1973). Absorption from the hydrophilic polyethylene glycol suppository base definitely occurs at a faster rate than was observed for hexobarbital from a lipophilic base (Chapter 1, Section III). It also follows from the relative bioavailability calculations that the polyethylene glycol base is able to deliver vinylbital to the blood to a satisfactory extent, since a 200 mg dose rectally yields blood levels in the same range as 150 mg orally. Preliminary in vitro experiments revealed that the dissolution rate of vinylbital from the present dosage forms was very rapid: within half an hour 70% had dissolved when the tablet was studied, whereas 85% had dissolved with the suppository (Levy beaker method; 37° C; 500 ml 0.1 N HCl). It is doubtful however whether this in vitro method has any predictive value for the in vivo process of rectal absorption, as the amount of fluid in vitro far exceeds the actual situation.

Elimination

The half-life of vinylbital in man is quite long with respect to its intended duration of action in hypnotic drug therapy. Hofmann (1971), who measured vinylbital blood levels after oral administration of 300 - 450 mg vinylbital to three healthy volunteers, observed that the duration of hypnotic - CNS depressant action lasted from 18 - 33 h. Each subject felt fit again when the blood concentration had decreased to about 2 mg/l. As already mentioned, in the present study the subjects felt a strong tendency to sleep at the time when the plasma concentration exceeded 2 mg/l. They slept mostly for a few hours - while they could easily be wakened - then took some food and went back to their daily work. All of them, except subject P.K., reported feelings of drow-

siness during the rest of the day, both after having received the tablet as well as after the suppository. It is striking that in subject P.K. the plasma concentrations were the lowest of all, and apparently below the minimal effective level. The subjects who participated in the study of repetitive vinylbital administration at night, complained of drowsiness the next morning, a few days after the beginning of the study. Plasma concentrations close to 3 mg/l were measured during these periods. It may be concluded that a level of 2 mg/l is a critical one, with respect to the appearance of feelings of CNS depression. Especially in the case of subjects with a low body-weight the plasma concentration remains a long time above this level after a 9 h night's rest (Fig. 1 and 2). It is possible that a lower concentration is required for the induction of sleep at night. This is substantiated by the fact that after administration of one BYKONOX tablet Jovanovic (1967a) noticed an insleep time of 17 - 27 min in his patients. By this time plasma concentrations have reached values of around 1 mg/l. For this reason, it might be recommendable to give a lower dose than 150 mg, when vinylbital is chosen for the treatment of insomnia.

Although vinylbital contains a chiral centre, the elimination phase in all volunteers could be described by a single first-order process. There were no indications for a biphasic decay of the plasma concentration, with a possible exception of subject G.V. after rectal administration. However, the latter curve could also be fitted by first order kinetics and this was preferred for reasons of uniformity. At present it is assumed that the enantiomers of vinylbital in man are eliminated with approximately the same rate, or at least that a possible difference is in the order of magnitude that there cannot be differentiated between in our experiments.

Elimination of vinylbital occurs to the greatest extent by metabolism, since only a small amount of the drug was excreted in the urine unchanged. Geldmacher - von Mallinckrodt et al. (1968; ib. 1971) identified two metabolites in human urine: 5-(1-methyl-3-hydroxybutyl)-barbituric acid (complete vinyl-group removed, which is the result of a rarely occurring C-dealkylation) and 5-vinyl-5-(1-methyl-3-hydroxybutyl)-barbituric acid. Of the dose administered 8 - 45% was excreted in the form of these two metabolites. These and other possible metabolites of vinylbital have been synthesized by Preuss and Müller (1968), but no definite identification of other metabolites in urine has been published so far.

With respect to the true values of the total body clearance constant (k_{el}) and the apparent volume of distribution (V_f) of vinylbital in man, it is important to realize that a smaller bioavailability (F) results in higher values for V_f/F and k_{el}/F. The smaller values of the two, in the same individual, are the closer to the true V_f and k_{el}. These have been calculated per kg body-weight (Table II)

and it appears that V_f/F per kg is quite constant among the subjects. If a linear relationship between body-weight and V_f is assumed, it is likely that the average value of 0.74 l/kg is a realistic one and that bioavailability now approaches 100%. This is true also for k_{el}, which is then approximately 0.36 ml/min/kg. The apparent volume of distribution of vinylbital in man is smaller than of hexobarbital, which is in agreement with its lower lipophility. Also, the clearance constant is much smaller compared to hexobarbital, indicating a less efficient drug - metabolizing enzyme interaction. In this respect the lower lipophility might also be a factor to be considered (Hansch, 1972). The results in man are consistent with the findings in rats, because van Kroonenburgh (1971) found a much lower clearance for vinylbital than Alvarez (1971) did for racemic hexobarbital.

Repetitive administration

Hypnotic drugs are taken frequently for longer periods of time and it is important therefore to obtain information of plasma levels during repetitive administration. Barbiturates in general, are considered to be potent enzyme inducers, also with respect to the induction of their own metabolism (Remmer, 1972). In this study on vinylbital, however, no such induction could be demonstrated despite its own extensive metabolism and persistence in the body (Fig. 6). To what extent vinylbital is incapable of increasing the rate of metabolism of other drugs, especially when it is given for longer periods of time, cannot be predicted and awaits further investigation.

In Fig. 7 the plasma concentration profile during repetitive vinylbital administration has been simulated (subject J.H.), assuming a constant half-life of 18 h. Also the theoretical curve is given, based upon the first single administration of the drug. It can be observed that the actual plasma concentrations are lower than the theoretical ones. This might be due to a lower bioavailability of vinylbital when it is taken at night, caused by the fact that the subjects are not fasting in that situation. After 3 - 4 days the equilibrium (steady state) situation has been reached. Following this, the lower and upper concentrations per dosage interval are about the same: the concentration fluctuates between discrete values. This observation is in agreement with the theory that an equilibrium state is reached during chronic administration, after approximately four times the half-life of the drug (van Rossum, 1971). It is evident that the plasma concentration during day-time varies from 3.5 to 2.2 mg/l, which is undesirably high when no day-time sedation is required. If, however, sedative therapy is the main objective of vinylbital administration, then a lower dose (e.g. 50 mg) twice

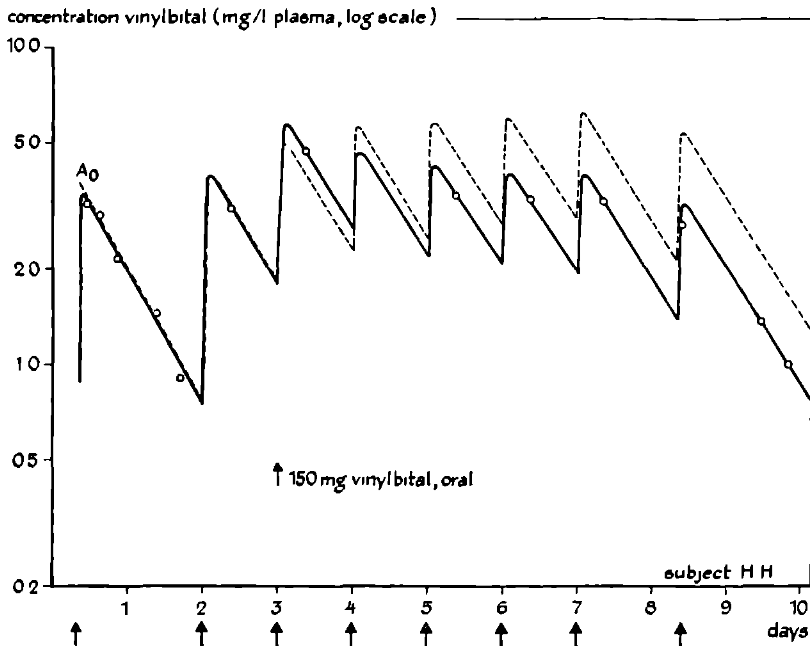

FIG. 7. Simulation of the plasma level profile during repetitive vinylbital administration, using the data of Fig 6 (solid line) It was assumed that peak levels were reached after 1 5 h and that the elimination half-life of 18 h remained constant throughout the trial. The dotted line represents the theoretical plasma level profile, based upon the first single administration of the drug. To the concentration at the end of each dosage interval a value equal to A_0 (3 7 mg/l) was added to obtain the curve for the next interval. The actual plasma concentrations remain below the theoretical ones and this might be due to a lower bioavailability of the drug when it is taken at night.

daily, would be more appropriate In this case a lower steady state level with less fluctuations would result, which is desirable in sedative drug therapy.

CONCLUSIONS

From the pharmacokinetic point of view, vinylbital cannot be considered as a suitable drug for the treatment of insomnia in non-bedridden persons. The long half-life of 17 - 32 h, especially in relation to a dose of 150 or 200 mg and a minimal effective plasma level of 2 mg/l, causes a high risk of drowsiness the

next morning. This is a factor that should be given serious consideration. When day-time sedation is required, as is often the case in hospitalized patients, vinylbital could be used beneficially, as the drug seems to have satisfactory pharmacodynamic properties (Jovanovic, 1967a; ib. 1967b).

The tablets are generally more suitable for sleep induction than the suppositories, as the absorption rate from the upper part of the gastrointestinal tract is higher. There are situations, however, where oral administration meets with difficulties; in that case the suppository could be used. Also in sedative drug therapy the suppository is a suitable dosage form, however then the dose should be diminished.

SUMMARY

The pharmacokinetics and relative bioavailability of vinylbital after oral and rectal administration were studied in man. Tablets (BYKONOX), containing 150 mg vinylbital, were used for the oral experiments and suppositories (SUPPOPTANOX, polyethylene glycol base), containing 200 mg vinylbital, were used for rectal administration. Six volunteers participated on two occasions in this study and received both preparations. Vinylbital plasma concentrations were determined at regular intervals after drug administration.

Absorption and elimination of vinylbital appeared to occur according to a single first-order process and the plasma concentrations were fitted by computer according to the equation intrinsic to the one-compartment open model after oral or rectal administration. The lag time was shorter for the suppository than for the tablet, whereas the absorption rate was faster for the tablet (mean absorption half-life 0.24 h compared with 0.64 h for the suppository). The elimination half-life of vinylbital varied from 17.6 to 33.5 h, with a mean value of 23.5 h for oral administration and 23.8 h for rectal administration. The half-lives were not considerably different on the two occasions for the individual volunteers. The average bioavailability of vinylbital for the suppository, relative to the tablet, was approximately 93%. Drowsiness was experienced by all volunteers, after receiving both dosage forms, and persisted for the remainder of the day.

Three volunteers collected their urine during 3 - 4 days after administration of a tablet. Unchanged vinylbital was determined and approximately 1.6% of the administered dose was excreted as unchanged drug.

Two volunteers participated in a study of repetitive vinylbital administration, during which a tablet was administered each night. Plasma concentrations were determined during and after repetitive dosing. Substantial accumulation of the drug in the body was not observed, although a relatively high plasma concentration was monitored during the day. The half-life of vinylbital was the same prior to and after the experiments of repetitive administration.

It has been concluded that the half-life of vinylbital is rather long with respect to its intended duration of action in the treatment of insomnia. Only when sedation is required during the day can vinylbital be used beneficially.

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HEPTABARBITAL AND HEPTABARBITAL SODIUM ORAL ADMINISTRATION

INTRODUCTION

Heptabarbital was introduced into hypnotic drug therapy in 1942 and it is available under the trade name MEDOMIN (tablets containing 200 mg of active substance). This preparation has been evaluated in several trials and it was shown to be effective in the treatment of insomnia (e.g., Steininger, 1953; Kotsovsky, 1954; Lienert, 1954; Brusca, 1955; Weithaler and Biedermann, 1960; Lienert, 1956; Bauer and Reckendorf, 1956; Ainley, 1957; Gillhespy, 1960; General Practioner Research Group, 1968). Heptabarbital is regarded as an intermediate-acting barbiturate with mild sleep maintaining properties (Martindale, 1972; Lienert, 1956). An important observation from many of the above studies has been that hardly any residual effects became apparent the following morning, after administration of the usual oral dose of 200 - 400 mg heptabarbital the previous night. This may indicate that the drug is rapidly inactivated in the body. However, this supposition has not been substantiated yet, since the half-life in man is not known.

In view of the general lack of information concerning the pharmacokinetics of heptabarbital in man, it was decided to investigate its rate of absorption and elimination after oral administration of MEDOMIN tablets. Preliminary experiments revealed that the rate of heptabarbital absorption from the tablets was relatively slow and for this reason heptabarbital sodium was included in the study. The sodium salt is very soluble in water and it was expected that this would cause an increased absorption rate, as was shown in the case of hexobarbital sodium (Chapter 1, Section III). The tablets as well as the sodium salt (in hard gelatine capsules) were administered to the same human volunteers, and this allowed the estimation of individual relative bioavailability.

Furthermore, plasma levels during repetitive heptabarbital administration were determined in order to ascertain whether accumulation of the drug in the

body occurred and also to determine whether the half-life of the drug would remain unchanged.

METHODS

The MEDOMIN tablets were obtained from CIBA-GEIGY, Arnhem, The Netherlands. The heptabarbital content was determined at the Dutch Pharmacist's Laboratory, The Hague, by potentiometric titration with alkali (0.1N) in a nitrogen atmosphere. An average content of 203.5 mg (101.8%) was found, with a variation coefficient of 3.3%. Heptabarbital sodium which is not commercially available, was prepared by treating the free acid with an equimolar quantity of sodium hydroxide solution and precipitating the sodium salt with diethyl ether. The heptabarbital sodium content of the white crystalline product obtained was 98.5%, determined by potentiometric titration with hydrochloric acid (0.1N).

Seven healthy male volunteers, ranging in age from 19 - 25 years and in body-weight from 55 - 85 kg, participated in the study. They had received no regular medication during the 4 weeks preceding initiation of the experiments. After an overnight fast, at 9 a.m., the subjects were given 200 mg heptabarbital as a MEDOMIN tablet or 218 mg heptabarbital sodium (this amount is equivalent to 200 mg free acid), in a hard gelatine capsule without additives, together with 150 ml water. They were requested to remain in an upright position for 15 min and then to lie down for at least 3.5 h. This procedure was undertaken in order to simulate as closely as possible the situation of taking a hypnotic drug and going to bed. No food, fluid or tobacco was allowed for at least 3.5 h after drug administration. Blood samples (5 ml) were taken from a forearm vein, usually at the following times: 20 and 40 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after drug administration. Every subject received both preparations with an interval of at least 4 weeks in a random sequence. The time schedule was the same for both trials.

Four of the volunteers collected their urine during 2 days after administration of a MEDOMIN tablet. Heptabarbital concentration in plasma and in urine was determined by gas chromatography, as described in Chapter 6, Section II.

Four volunteers participated in a study of repetitive heptabarbital administration. On the first day of the experiment the half-life of the drug was determined, by administering a MEDOMIN tablet in the morning at 9 a.m. and collecting blood samples at regular intervals. Beginning on the second night, the volun-

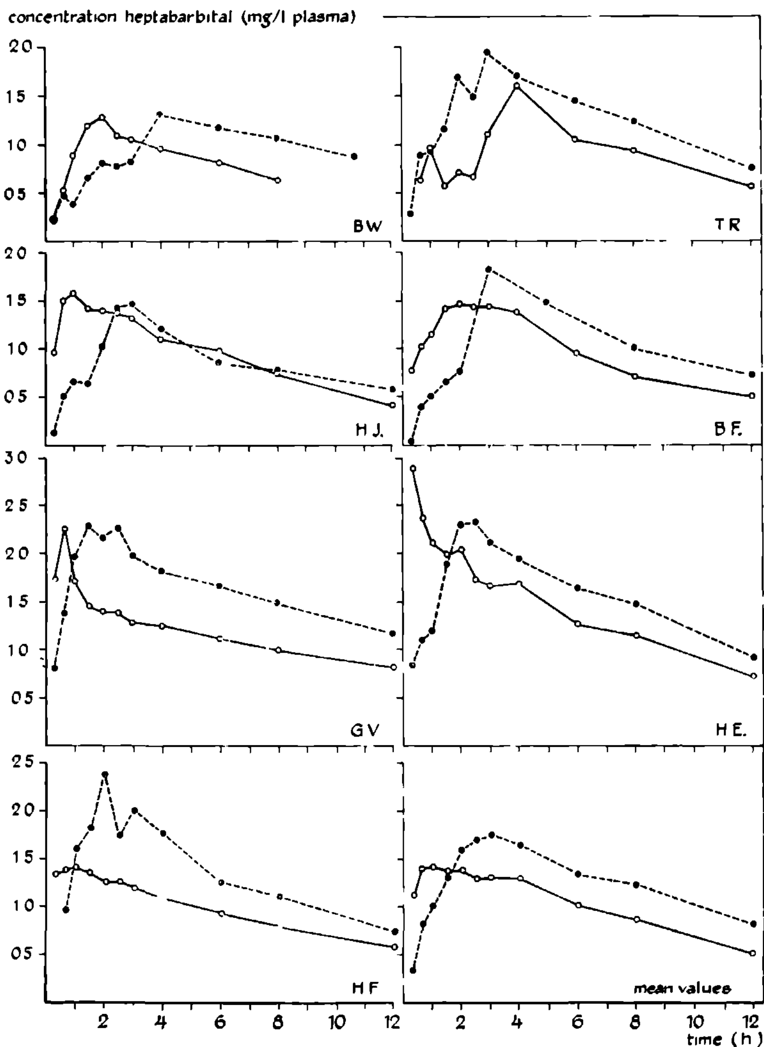

FIG. 1. Plasma concentration curves after oral administration of 200 mg heptabarbital as a MEDOMIN tablet (dotted line) and 218 mg heptabarbital sodium in a hard gelatine capsule (solid line) to seven healthy male volunteers. Also, the average curves are given. Note the more rapid absorption with the sodium salt in almost every volunteer. Also note that the area under the plasma concentration curve is generally smaller with the sodium salt, indicating a lower bioavailability of heptabarbital when administered as the sodium salt (Table II).

teers took a MEDOMIN tablet with 150 ml water. Usually the next morning, nine hours after drug intake, a blood sample was taken and the heptabarbital plasma concentration was determined. After eight (two volunteers) or ten days (two volunteers), heptabarbital was again given in the morning, in order to determine the half-life of the drug after repetitive administration. In Fig. 5 the time schedule of drug intake and blood sampling is indicated.

RESULTS

Rate of absorption and elimination

In Fig. 1 all of the individual plasma concentration curves are shown, which were obtained after oral administration of 200 mg heptabarbital as free acid (MEDOMIN) and 200 mg heptabarbital as sodium salt to each volunteer. It is evident that the absorption of heptabarbital was quite slow, e.g. it took 1.5-4 h before the maximum plasma concentrations were attained, which were in the range of 1.3 - 2.4 mg/l. When heptabarbital was administered as the sodium salt a more rapid rise of the plasma concentration occurred; peak concentrations were reached after $^1/_3$ - 2 h, except for volunteer T.R. The maximum plasma concentrations varied from 1.3 - 2.8 mg/l (Table I). CNS depression was experienced by all subjects, 1.5 - 2.5 h after receiving the MEDOMIN tablet and approximately 1 h after receiving the sodium salt. Usually heptabarbital plasma concentrations had exceeded 1 mg/l when drowsiness was experienced and in some cases these were substantially higher. For instance, when early peak concentrations with the sodium salt had been reached, it was noticed that CNS depression became apparent some time after the peak level time. This indicates that a latency time exists for heptabarbital with respect to penetration into the brain. The physicochemical properties of a drug determine its rate of penetration through the blood-brain barrier (Goldstein et al., 1969) and it has been shown that for the less lipophilic barbiturates, to which heptabarbital belongs, there is a latency with respect to the onset of anaesthetic action after i.v. administration (Butler, 1942). Despite the high amount of drug that is carried by the bloodstream to the brain immediately after i.v. injection, it takes some time before an effective brain concentration has been reached. Obviously, after oral administration a similar phenomenon may only become apparent if absorption is very rapid in comparison to distribution of the drug into the brain. In such a case a relatively rapid decrease in plasma concentration directly after termination of absorption may be observed, which is due to the distribution of heptabarbital from plasma into tissues (subjects G.V. and H.E.).

TABLE I. Absorption characteristics of heptabarbital and heptabarbital sodium after oral administration to the same healthy volunteers.

Subject	Body-weight (kg)	Chemical form[1]	Dose[2] (mg)	Dose/kg (free acid; mg)	t_{max}[3] (min.)	C_{max}[3] (mg/l)
B.F.	70	acid	200	2.86	180	1.84
		salt	218	2.86	120	1.48
T.R.	68	acid	200	2.94	180	1.96
		salt	218	2.94	240	1.61
B.W.	85	acid	200	2.35	240	1.31
		salt	218	2.35	120	1.28
H.J.	75	acid	200	2.67	180	1.46
		salt	218	2.67	60	1.59
G.V.	71	acid	200	2.82	90	2.29
		salt	218	2.82	40	2.26
H.F.	80	acid	200	2.50	120	2.38
		salt	218	2.50	60	1.42
H.E.	55	acid	200	3.64	150	2.32
		salt	218	3.64	20	2.84
Mean values	72	acid	200	2.83	163	1.94
		salt	218	2.83	96	1.78

[1]) Heptabarbital as free acid was administered in the form of a MEDOMIN tablet. Heptabarbital sodium was administered in hard gelatine capsules, without additives.

[2]) 218 mg of the sodium salt is equivalent to 200 mg of heptabarbital as free acid.

[3]) t_{max} refers to the time after which the maximum plasma concentration (C_{max}) is reached.

In many of the present experiments the absorption phase of heptabarbital could not be described accurately by first-order kinetics. Absorption was sometimes irregular or occurred in other cases so rapidly, that sufficient data were not available during the absorption phase to permit first-order fitting. As a measure for the rate of absorption, therefore, the time after which the maximum plasma concentration was reached, is given (Table I). This is a common procedure used in biopharmaceutical studies (Ritschel, 1972). In contrast with

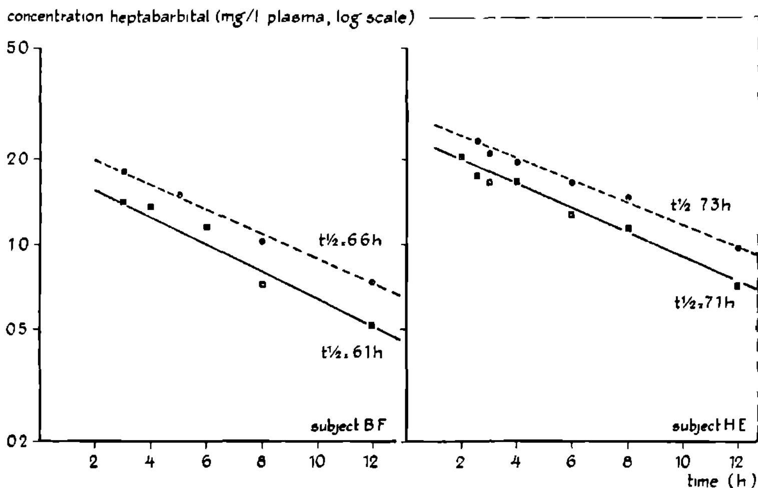

FIG. 2. Plasma concentration curves on semi-logarithmic scale representing the elimination phase of heptabarbital after oral administration of a MEDOMIN tablet (dotted line) and heptabarbital sodium (solid line) to the same volunteers. These data are from the same experiments as in Fig. 1. Note that the half-lives in a particular volunteer are approximately the same on different occasions (interval of more than 4 weeks).

absorption, elimination of heptabarbital may be described by a first-order process, since on all occasions straight lines were obtained for the decrease in concentration after plotting these data on a semi-logarithmic scale (Fig. 2). The plasma half-lives varied from 6.1 - 11.2 h, which is a relatively short period for the barbiturates (Table II). Also, it was observed that the half-lives of the barbiturate were about the same for each individual at both occasions (Fig. 2; Table II). The small differences measured may not be meaningful since they are in the same order as the experimental error.

Relative bioavailability estimation

With respect to the bioavailability of heptabarbital the following equation applies, assuming linear kinetics:

$$F \times D = k_{el} \cdot \int_0^\infty C_p \, dt = k_{el} \cdot AUC$$

where F = bioavailability ($0 \leqslant F \leqslant 1$), D = dose administered (mg), C_p = plasma concentration (mg/l), k_{el} = total body clearance constant (ml/min), AUC = area under the plasma concentration curve from $t = 0$ to $t = \infty$ (mg.h/l). The clearance constant can only be estimated accurately after i.v. administration and therefore absolute bioavailability cannot be determined if only data of oral administration are available. However, the cross-over design of the present investigation permits an estimation of the relative bioavailability of heptabarbital to be made for each individual, assuming that k_{el} had the same value on both occasions of drug administration. Evidence for this supposition lies in the fact that the half-life had almost the same value on both occasions for the same individual. The following equation now applies, taking the tablet (free acid) as the reference dosage form:

$$F_{rel} = \frac{AUC_{free\ acid}}{AUC_{salt}}$$

If the AUC is not determined to $t = \infty$, then a correction should be made to include the undetermined AUC. This is possible by assuming that at the last data point, at time t′, absorption is complete. Using Dost's law of corresponding areas (Dost, 1968), the infinite area is given by:

$$\int_0^\infty C_p\,dt = \int_0^{t'} C_p\,dt + \int_{t'}^\infty C_p\,dt = AUC\Big/_0^{t'} + C_p^{t'} \cdot \tau_{el}$$

where τ_{el} is the elimination time constant ($\tau_{el} = 1.44 \times t_{1/2}$) and $C_p^{t'}$ is the last plasma concentration measured at time t′. The AUC's from $t = 0$ to $t = t'$ ($AUC\Big/_0^\infty$), in the present experiments, were determined by weighing the corresponding areas under the plasma concentration curves, usually up to 12 h after administration. The remaining area was calculated by multiplying the concentrations at 12 h by the average τ_{el}. In Table II the results are given for the seven volunteers. In all but one case (subject B.W.) the sodium salt showed a lower bioavailability than the free acid.

If an average bioavailability of 100% is assumed for the tablets, then the average clearance constant and apparent volume of distribution in man can be estimated. When the average plasma concentration curve for the tablet was plotted on semi-logarithmic scale, it became apparent that the absorption phase could be described by first-order kinetics (Fig. 3). The parameters deduced

TABLE II. Elimination half-lives and relative bioavailability of heptabarbital after oral administration of heptabarbital as free acid and as the sodium salt to the same healthy volunteers.

Subject	Chemical form	$t_{1/2}$ (h)	Average τ_{el} (h)	AUC 0 - 12 h (mg.h/l)	Conc. 12 h (mg/l)	AUC 0 - ∞ (mg.h/l)	F_{rel} %
B.F.	acid	6.6		12.7	0.73	19.4	100
	salt	6.1		11.5	0.51	16.2	81
		$\overline{6.4}$	9.2				
T.R.	acid	6.5		15.2	0.74	22.1	100
	salt	6.2		11.0	0.55	16.1	73
		$\overline{6.4}$	9.2				
B.W.	acid	8.4		11.1	0.76	20.1	100
	salt	7.9		7.0(8h)	0.63(8h)	14.4	72
		8.2	11.8				
H.J.	acid	6.2		10.1	0.42	13.7	100
	salt	6.2		11.2	0.60	16.4	119
		$\overline{6.2}$	8.6				
G.V.	acid	11.0		19.4	1.17	37.9	100
	salt	11.2		13.7	0.82	26.7	70
		$\overline{11.1}$	15.8				
H.F.	acid	7.8		15.4	0.74	23.8	100
	salt	8.0		11.2	0.58	17.8	75
		7.9	11.4				
H.E.	acid	7.3		18.2	0.92	27.9	100
	salt	7.1		16.5	0.72	23.9	86
		$\overline{7.2}$	10.4				
Mean values	acid	7.7		14.6	0.78	23.6	100
	salt	7.5		12.5	0.63	18.8	83
		$\overline{7.6}$	10.9				

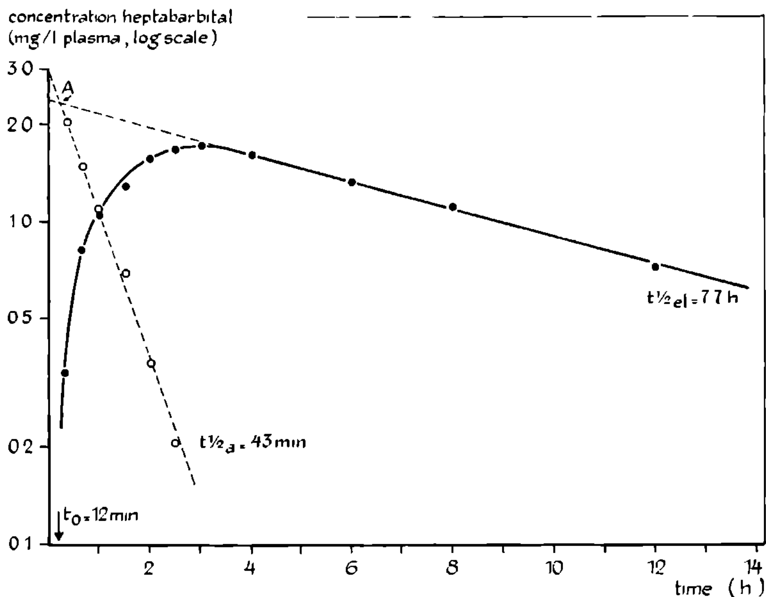

FIG. 3. Mean plasma concentration curve of heptabarbital on semi-logarithmic scale, obtained after oral administration of a MEDOMIN tablet to seven healthy volunteers. For the individual curves and for the mean curve on linear scale, see Fig. 1. The average absorption phase and elimination phase can be described by first-order kinetics. The half-life of absorption has been estimated by means of the subtraction method (dotted line). From the experimental parameters the average volume of distribution and clearance constant of heptabarbital in man can be estimated (see text).

from this curve were: t_0 (12 min), A (2.35 mg/l), $t_{1/2a}$ (43 min), $t_{1/2el}$ (7.7 h). Furthermore k_{el} and V_f were calculated (Section I, Chapter 2) and found to be 138 ml/min and 94 l respectively.

Renal excretion

Heptabarbital was excreted unchanged in the urine to a very small extent. For the four volunteers, who collected their urine after the administration of 200 mg heptabarbital (MEDOMIN tablet) during a period of about two days, cumulative urinary excretion amounted to 0.16 - 0.30% of the dose administered. The excretion plateau was reached after 26 - 40 h had elapsed, which is

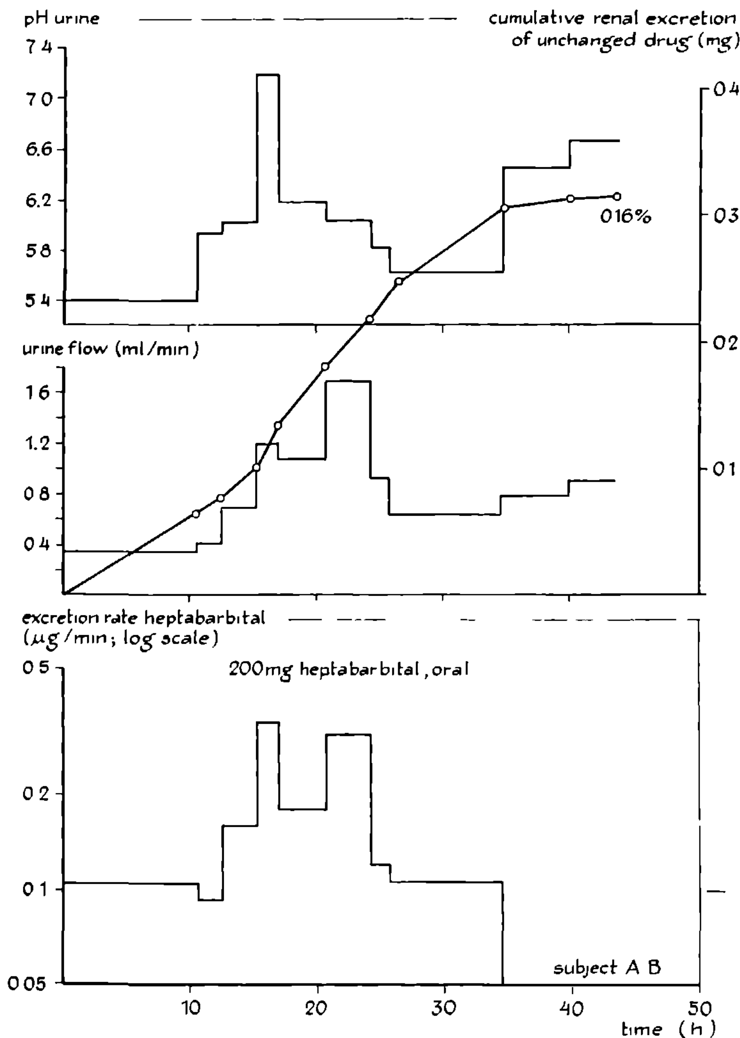

FIG. 4. Urine pH, urine flow, cumulative renal excretion of unchanged drug and renal heptabarbital excretion rate after oral administration of 200 mg heptabarbital. The excretion rate increases with increasing urine pH and increasing urine flow. Only a very small fraction of the administered dose is excreted into the urine as unchanged drug.

consistent with the short plasma half-life of heptabarbital. The drug could be measured unchanged up to this time (Fig. 4) which is in contrast to the results

of Gilbert et al. (1973) who detected unchanged drug only in the first 8 h urine batch after oral administration of 400 mg heptabarbital to a human volunteer. The same authors identified by combined gas chromatography – mass spectrometry, two heptabarbital metabolites in urine, the 3'-hydroxy – and the 3'-keto-derivative. The formation of the latter compound had previously been demonstrated by Pulver (1943) and Bernhard and Bickel (1957). The metabolites mentioned, however, account for only part of the total amount of heptabarbital administered. Nothing is known about the further biodegradation of this drug. It can be observed from Fig. 4 that the renal excretion rate of heptabarbital fluctuates considerably and appears to be dependent on urine pH and urine flow. As a consequence of the relatively low pK_a value of the drug (7.45) (Doornbos and de Zeeuw, 1969), the excretion rate is found to increase when the urine pH exceeds 7. This is important in the management of heptabarbital overdosage, where alkalinization of the urine may be beneficial.

Repetitive heptabarbital administration

After repetitive heptabarbital administration the half-lives in three of the four subjects did not change significantly (Table III). The possibility of the development of enzyme induction must, however, be taken into consideration, since in one subject (F.H.) the half-life had clearly decreased. Due to the short half-life, no accumulation occurred during repetitive heptabarbital administration (Fig. 5). The concentrations, measured each morning 9 h after drug intake, were similar whereas with volunteer F.H. with the shortening half-life, these values were inclined to decrease after a few days. In Fig. 6 the theoretical plasma concentration profile has been simulated (volunteer B.F.). This is based upon the curve which was obtained after the first single dose, assuming

TABLE III. Elimination half-lives of heptabarbital before and after repetitive administration of 200 mg each night.

Subject	$t_{1/2}$ (h)		Number of doses
	Before	After	
M.W.	8.2	7.8	10
B.F.	5.8	6.0	10
G.V.	10.5	10.8	8
F.H.	7.1	4.6	8

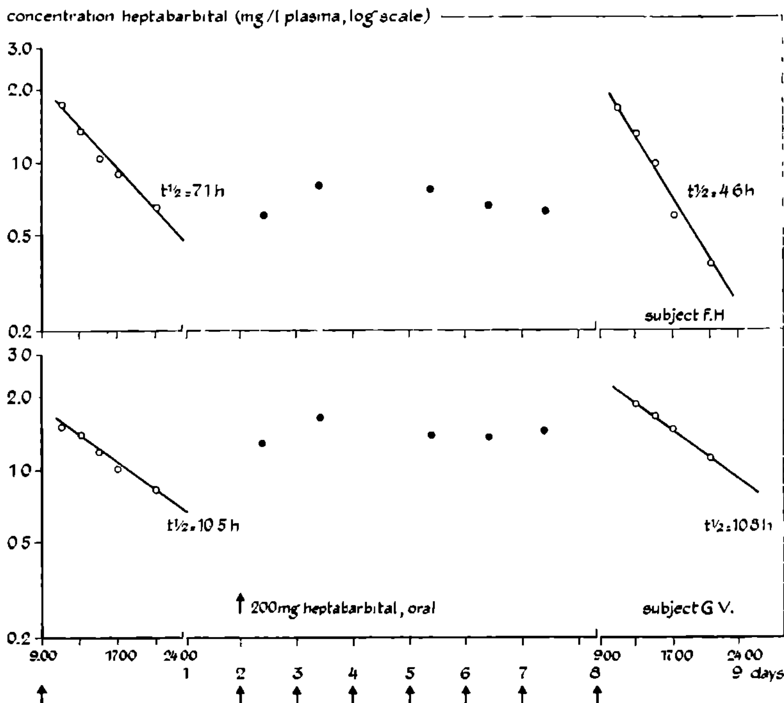

FIG. 5. Plasma concentrations of heptabarbital during and after repetitive heptabarbital administration to two volunteers. During the first and the last day of the experiment the half-life of the drug was determined. From the second night on a MEDOMIN tablet was taken each night and usually the next morning (9 h later) the plasma concentration was measured. Note that these concentrations were always about the same. Also note the shortened half-life of the drug in one of the volunteers at the end of the trial, indicating the development of enzyme induction.

a constant half-life of 6.4 h. It may be observed that the experimental data closely fit this curve. With the half-life of 6.4 h and a dosage interval of 24 h, only 5 - 10% of the previously administered dose remains in the body before the next dose is taken. This is such a small amount, that in practice each dose can be considered as a repetition of the first single dose.

DISCUSSION

Prior to the passage of a drug through a biological membrane, dissolution in the fluids surrounding that membrane must occur (Garrett, 1971; Benet,

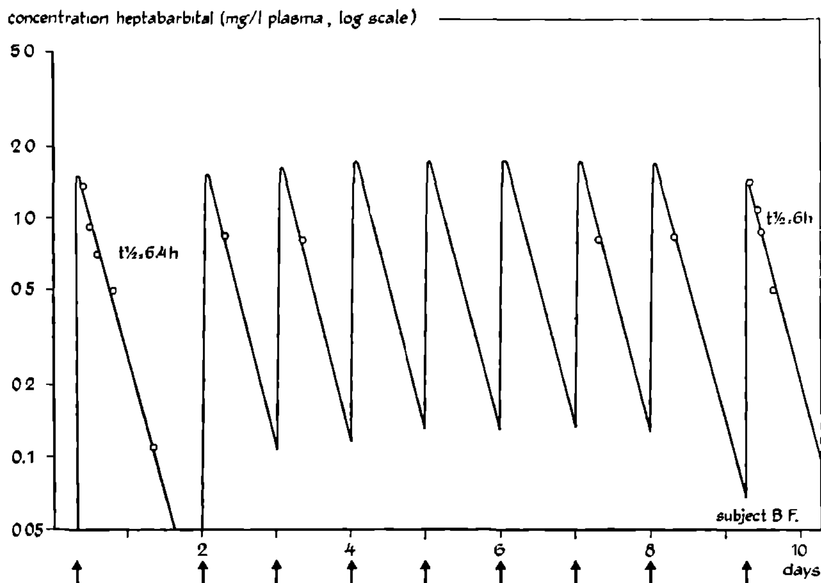

FIG. 6. Theoretical plasma level profile during repetitive heptabarbital administration, based upon the curve obtained after the first single dose. The arrows indicate drug intake (200 mg every 24 h). The plasma concentrations determined 9 h after drug administration and the experimental data after the last dose, closely fit the calculated curve.

1973). It has been demonstrated that the rate of absorption of several drugs, which are slightly water-soluble, is limited by their solubility, e.g. griseofulvin (Atkinson et al., 1962) and prednisone (Levy et al., 1964). The slow absorption of heptabarbital, when administered as the free acid, is probably due to its poor water-solubility, which is less than 0.5 g/liter (Martindale, 1972). Preliminary in vitro experiments (Levy beaker method; 37° C; 80 r.p.m.; 500 ml 0.1N HCl) have demonstrated that the dissolution of heptabarbital from the tablets is greater than dissolution of heptabarbital as a bulk substance (the same material as used for tablet compounding); 25% of the tablet form and 5% of the bulk substance had dissolved after 3 h. Usually, tablets contain a wetting agent (e.g. gelatine) which favours dissolution and thereby increases the rate of drug absorption. This was shown for secobarbital by Sjögren (1971). The present findings indicate that the absorption of heptabarbital is dissolution rate-limited when the drug is administered as the free acid.

Sodium salts of barbiturates are readily soluble in water, which is the explanation for the higher heptabarbital absorption rate of the sodium salt-containing capsules. In general, salts will show higher dissolution rates than the corresponding non-ionic drug at any pH, even though the final equilibrium solubility of the drug and its salt are the same (Higuchi et al., 1958). This is explained by the fact that if precipitation occurs, generally a suspension of fine particles results, which has the proper characteristics for rapid redissolution. This phenomenon apparently applies in the case of barbiturates, since their sodium salts will be precipitated in the acid medium of the stomach, but nevertheless a much higher absorption rate is observed than with the corresponding free acids (compare hexobarbital and hexobarbital sodium, Chapter 1, Section III). Sjögren et al. (1965) reported similar findings for pentobarbital and secobarbital absorption. The preliminary in vitro experiments on the heptabarbital sodium capsules showed a rapid dissolution of heptabarbital in 0.1N HCl; within 30 min 40% had dissolved, which almost corresponded to the saturation concentration.

The results on the bioavailability of heptabarbital revealed that the drug reaches the general circulation to a greater extent after administration as the free acid than as the sodium salt. This is an unexpected finding, since it is a common belief that a high dissolution rate favours high bioavailability (Nelson, 1961; Wagner, 1970; Garrett, 1971). Bioavailability problems most often arise with preparations which show relatively poor dissolution characteristics (Blake, 1971; Benet, 1973). Heptabarbital sodium, however, is rapidly absorbed (Fig. 1), which indicates that the dissolution rate of the drug in the gastrointestinal fluid is also high. As already discussed, the precipitation of the free acid in the stomach after heptabarbital sodium administration will for the greater part result in a suspension of fine particles, which readily redissolve. However, the possibility that also relatively gross crystalline particles are being formed, which do not redissolve completely, cannot be excluded. This would result in incomplete bioavailability. Other contributing factors may be that the gelatine of the capsule is interfering or that part of the precipitate is formed in a different crystalline form, with poor dissolution characteristics. Several examples are known of polymorphism influencing drug absorption (Haleblian and McCrone, 1969). Further experiments, in vitro and in vivo, are required to clarify the present findings.

The average half-life (7.6 h) of heptabarbital in man is relatively short, which is quite advantageous with respect to its intended duration of action in the treatment of insomnia. After a night's rest of 9 hours approximately 60 - 75% of the drug is inactivated. Thus, it is very likely that the plasma concentration

has decreased below the minimal effective level, if a 200 mg dose has been given. This level may vary from 1 - 2 mg/l, since the subjects who participated in the present study displayed evidence of lethargy at these plasma concentrations or higher ones. After a period of 4 - 6 h following drug administration, the volunteers expressed a general return to fitness. Lienert (1954) and Weithaler and Biedermann (1955) have shown that after oral intake of 200 - 400 mg heptabarbital at night, no residual effects were present the following morning. In our experiments with chronic heptabarbital administration, the volunteers did not complain of drowsiness after arising. Lienert (1956) noticed that the onset of hypnotic action with MEDOMIN tablets was relatively slow. Weithaler and Biedermann (1955) have concluded that MEDOMIN requires a certain „induction time" (1 - 2 h) before causing CNS depression. Furthermore, Fernandez-Guardiola et al. (1972), who performed a polygraphic study on hypnotic drugs in healthy volunteers, found that approximately 1 h after oral administration of a MEDOMIN tablet psychological changes became apparent. These findings are consistent with the slow absorption of heptabarbital from the tablets, as demonstrated in the present investigation. It must be concluded that heptabarbital as free acid is not capable of causing a rapid onset of hypnotic action. The sodium salt, however, although never studied clinically in this respect, may be expected to induce sleep more readily.

From the experiments of repetitive heptabarbital administration it is evident that there is a risk of enzyme induction with heptabarbital, since in one of the four subjects a shortened half-life was found. It was shown previously that repetitive administration of 400 mg heptabarbital resulted in a shorter half-life of coumarin anticoagulants in man when the drugs were taken concomitantly (Dayton et al., 1961; Aggeler and O'Reilly, 1969). The development of enzyme induction due to the influence of heptabarbital is somewhat surprising, since with the doses applied there is no persistence of a great amount of the drug in the body. Enzyme induction is often associated with compounds that accumulate in the body because of their long half-lives, e.g. butobarbital (Chapter 6, Section III), phenobarbital (Remmer, 1972). This author has remarked however, that, in principle, all lipid soluble organic substances are potential inducers. Also, it has been shown previously that enzyme induction in man is very inconsistent. Many people respond differently even when treated with the same compound at the same dose regimen (Cucinell et al., 1965). A similar finding was made in the present study, when only one of the four subjects developed enzyme induction. The risk of accelerated drug metabolism must be considered, when heptabarbital is used simultaneously with other drugs.

CONCLUSIONS

These pharmacokinetic studies reveal that heptabarbital may be regarded as a suitable drug for the treatment of insomnia. The relatively short half-life of 6 - 11 h in relation to a 200 mg dose, minimizes the risk of residual effects the following morning. During repetitive heptabarbital administration no accumulation of the drug occurs in the body. It should be considered, when other drugs are prescribed, that acceleration of drug metabolism may develop.

Since the absorption of heptabarbital from the MEDOMIN tablets is relatively slow, this preparation may be applied in instances where the maintenance of sleep is the primary prerequisite of hypnotic drug treatment. Heptabarbital is more rapidly absorbed when administered as the sodium salt, and this formulation is preferred for rapid sleep induction.

SUMMARY

The pharmacokinetics and relative bioavailability of heptabarbital and heptabarbital sodium were studied after oral administration. MEDOMIN tablets, containing 200 mg heptabarbital, and hard gelatine capsules, containing an equivalent amount of the sodium salt, were used. Seven healthy volunteers participated on two occasions in the investigation and received both preparations. Heptabarbital plasma concentrations were determined at regular intervals.

The absorption of heptabarbital from the tablets was quite slow, peak level times varied from 1.5 to 4 h. A more rapid absorption occurred when the sodium salt was administered, with peak level times between 1/3 and 2 h. In all cases the elimination of heptabarbital could be described by a single first-order process with an average half-life of 7.6 h (range 6.1 - 11.2 h). The half-life of the drug, for each individual, was about the same at the two trials. The relative bioavailability was estimated for each volunteer by comparing the areas under the plasma concentration curves. For the sodium salt an average bioavailability of 83% relative to the free acid was estimated. In some volunteers urinary excretion of unchanged heptabarbital was measured; cumulative excretion amounted to 0.16 - 0.30% of the administered dose. Four volunteers received a MEDOMIN tablet each night for eight or ten days; accumulation of heptabarbital during repetitive administration did not occur. In three volunteers the half-life of the drug, prior to and after these experiments, had not changed whereas in one volunteer the half-life had decreased from 7.1 to 4.6 h. The development of enzyme induction should be considered when heptabarbital is prescribed.

It was concluded that heptabarbital may be regarded as a suitable drug for the treatment of insomnia, since its half-life is rather short. Heptabarbital sodium may be used for sleep induction, whereas MEDOMIN tablets may be prescribed in cases where the maintenance of sleep is the primary prequisite of hypnotic drug treatment.

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BUTOBARBITAL
ORAL ADMINISTRATION

INTRODUCTION

Butobarbital, which was introduced in 1931, is one of the oldest barbiturates used in drug therapy. It is still widely applied in the treatment of insomnia, although its former popularity has decreased since the introduction of non-barbiturate hypnotics. The compound has been classified as an intermediate-acting barbiturate (Martindale, 1972), but this classification is controversial (Mark, 1969; Chapter 3, Section I). In several clinical trials butobarbital was found to be an effective hypnotic drug for sleep induction and sleep maintenance (e.g., Stewart, 1956; Hare, 1959; Lodge-Patch et al., 1960; Parsons, 1963; Zelvelder, 1971; Bond and Lader, 1972). This drug is also available under the trade name SONERYL (tablets containing 100 mg butobarbital). The usual oral dose is 100 - 200 mg at night.

In view of the paucity of knowledge regarding the pharmacokinetics of butobarbital in man, it was considered pertinent to investigate both the rate of absorption and the rate of elimination of this drug after oral administration. Furthermore, plasma levels during repetitive butobarbital administration were determined in order to see if accumulation of the drug in the body would occur and also to ascertain whether the half-life of the drug would remain unchanged.

METHODS

Butobarbital was obtained in bulk from Onderlinge Pharmaceutische Groothandel, Utrecht, through the Pharmacy of the St. Radboudhospital, Nijmegen. Hard gelatine capsules were loosely filled with 200 mg of this drug.

Five healthy male volunteers, ranging in age from 21 -25 years and in bodyweight from 66 - 81 kg, participated in the study. They had received no regular

medication during the 4 weeks preceding the initiation of the experiment. After an overnight fast, at 9 a.m., the subjects were given 200 mg butobarbital orally with 150 ml water. Initially, the volunteers were asked to remain in an upright position for 15 min and then to lie down for at least 3.5 h. This procedure was undertaken in order to simulate as closely as possible the situation of taking a hypnotic drug and going to bed. No food, fluid or tobacco was allowed for 3.5 h after drug administration. Blood samples (5 ml) were taken from a forearm vein, usually at the following times: 20 and 40 min an 1, 1.5, 2, 5, 8, 24, 48, 72 and 96 h after drug administration. Butobarbital concentration in plasma was determined by gas chromatography, as described in Chapter 7, Section II.

Approximately two weeks after the single dose experiment, four of the same volunteers participated in a study of repetitive oral butobarbital administration. Two of them received 200 mg butobarbital for three consecutive nights and the other two received 100 mg or 50 mg for nine consecutive nights. The subjects were instructed neither to take alcoholic beverages during the entire course of the experiment nor to drive a motor vehicle. Blood samples were taken usually 9 h after drug intake and the butobarbital plasma concentration was determined. After administration of the last dose blood samples were taken at regular times in order to determine the butobarbital plasma half-life after repetitive administration. In Table III the time schedule of drug intake and blood sampling is indicated.

RESULTS

The individual plasma concentration curves for the first 8 h after a single oral dose of butobarbital are shown in Fig. 1. Absorption was quite rapid, since a relatively high plasma concentration was attained 20 min after drug administration. Peak levels were reached between 40 min and 2 h; absorption apparently continued in subject R.C. for up to 8 h. CNS depression became evident in all volunteers after ½ - 1½ h, when the plasma concentration had exceeded 2 - 3 mg/l.

In Fig. 2, the plasma concentrations of the same volunteers are given on semi-logarithmic scale from 2 to 96 h. Butobarbital elimination can be described by a first-order kinetic process, because in all cases straight lines were obtained for the decrease in concentration. The half-lives deduced from these curves varied from 34 to 42 h. If it is assumed that absorption also is a first-order process, then the plasma concentration (C_p) can be described by the following equation (single-compartment model with absorption phase):

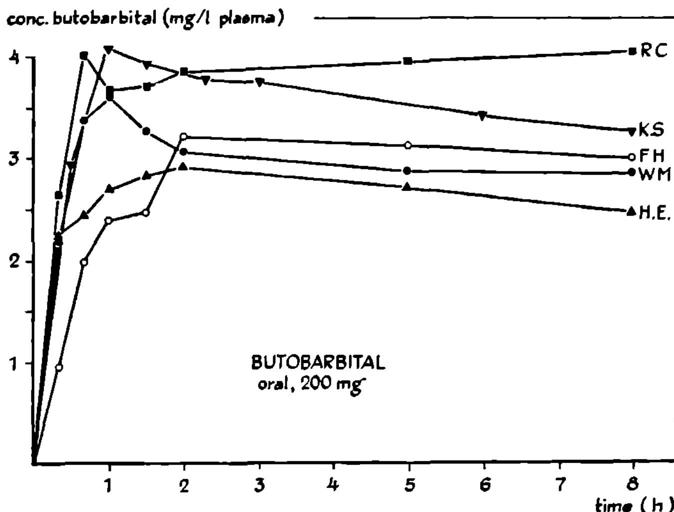

FIG. 1. Plasma concentration curves after oral administration of 200 mg butobarbital in a hard gelatine capsule to five healthy male volunteers (linear scale).

$$C_p = A \cdot (e^{-t/\tau_{el}} - e^{-t/\tau_a})$$

where τ_a and τ_{el} are the time constants of absorption and elimination respectively and A is the „zero-time concentration". The individual curves have been fitted according to the above equation (including correction for the lag time t_0) by means of the FARMFIT computer program; fitted parameters: τ_a, τ_{el}, A and t_0 (Table I). An example of a fitted and plotted curve is shown in Fig. 3. Good fits were obtained, although the relative errors in the absorption time constant and in the lag time were generally substantial. This is due most likely to the limited number of data points during the absorption phase. With reference to the volunteers R.C. and K.S., the relatively long lag time when compared with the very small τ_a does not seem to be realistic after perusal of the individual curves. In addition it was observed that for volunteers W.M. and H.E. the maximum plasma concentrations were higher than expected on basis of mono-exponential absorption and elimination (Fig. 3). This may indicate that in these cases absorption of the drug was more rapid than distribution into tissues. Thus, two-compartment kinetics may be more appropriate.

The apparent volume of distribution (V_f) and the clearance constant (k_{el}) of butobarbital in man, cannot be estimated accurately from the present

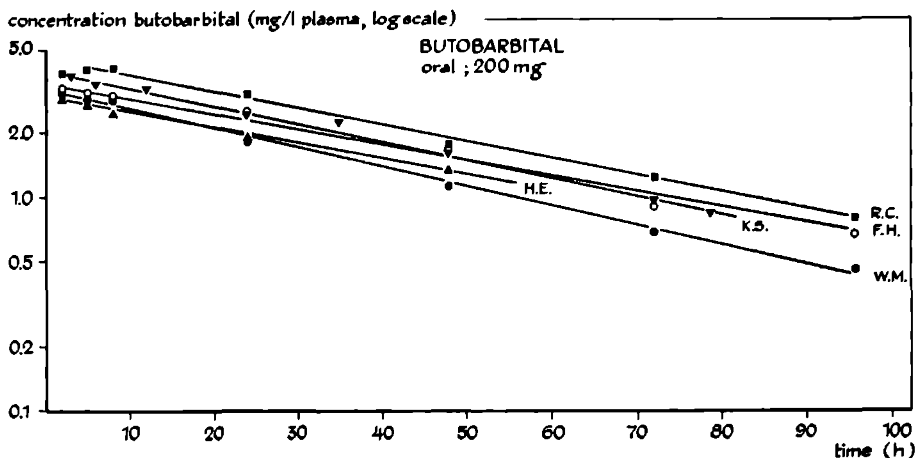

FIG. 2. Plasma concentration curves on semi-logarithmic scale of the same experiments as in Fig. 1. Note the long half-life of butobarbital in man (34 - 42 h).

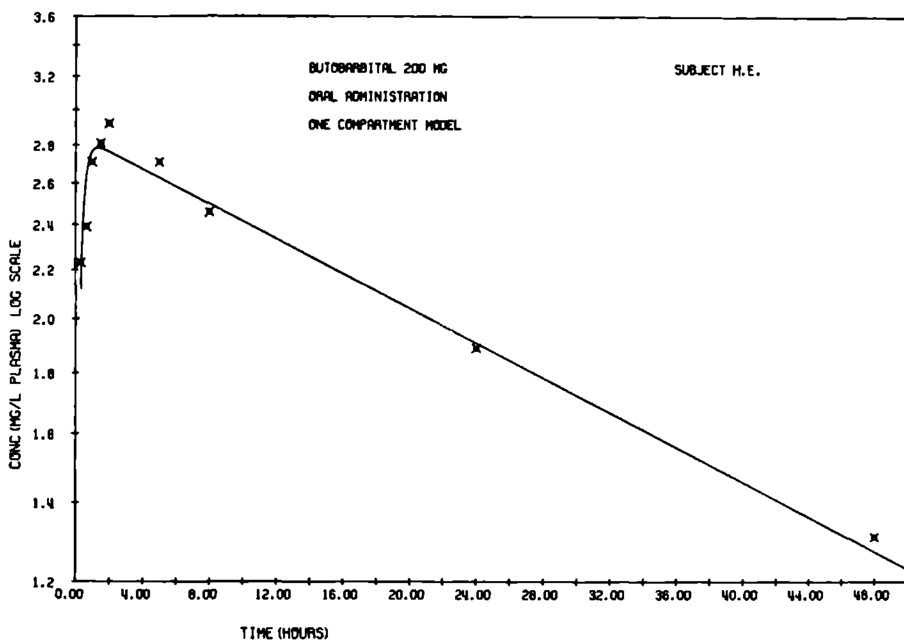

FIG. 3. Example of a fitted and directly computer plotted butobarbital plasma concentration curve. See Table I for parameter values.

TABLE I. Fitted pharmacokinetic parameters of butobarbital after oral administration.

Subject	Body-weight (kg)	Dose (mg)	Dose/kg (mg)	t_0 (h)	τ_a (h)	$t_{1/2a}$ (h)	τ_{el} (h)	$t_{1/2el}$ (h)	A (mg/l)
R.C.	81	200	2.47	0.23	0.10	0.07	57.7	40.3	4.05
K.S.	66	200	3.03	0.27	0.04	0.03	49.8	34.8	4.01
F.H.	81	200	2.47	0.05	0.85	0.60	54.7	37.2	3.56
W.M.	70	200	2.86	0.00	0.30	0.21	48.0	33.6	3.25
H.E.	76	200	2.63	0.00	0.24	0.17	59.3	41.5	2.86
Mean values		200	2.69	0.11	0.31	0.22	53.9	37.5	3.55

TABLE II. Calculated pharmacokinetic parameters of butobarbital after oral administration.

Subject	Body-weight (kg)	$\frac{V_f}{F}$ (l)	$(\frac{V_f}{F})$/kg (l)	$\frac{k_{el}}{F}$ (ml/min)	$(\frac{k_{el}}{F})$/kg (ml/min)
R.C.	81	49.5	0.61	14.3	0.18
K.S.	66	50.0	0.76	16.7	0.25
F.H.	81	57.1	0.70	18.0	0.22
W.M.	70	62.0	0.89	21.5	0.31
H.E.	76	70.2	0.92	19.7	0.26
Mean values		57.8	0.78	18.0	0.24

experiments. After oral administration the bioavailability F remains unknown. On the other hand, there is no reason to assume that absorption has not been complete, since the drug was administered in a loosely filled capsule on an empty stomach. Assuming complete absorption ($F = 1$), the values for V_f and k_{el} on the average in man may be close to 58 l and 18 ml/min respectively (Table II). The volume of distribution is in the same order of magnitude as was found for vinylbital (Chapter 4, Section III) and this is consistent with the

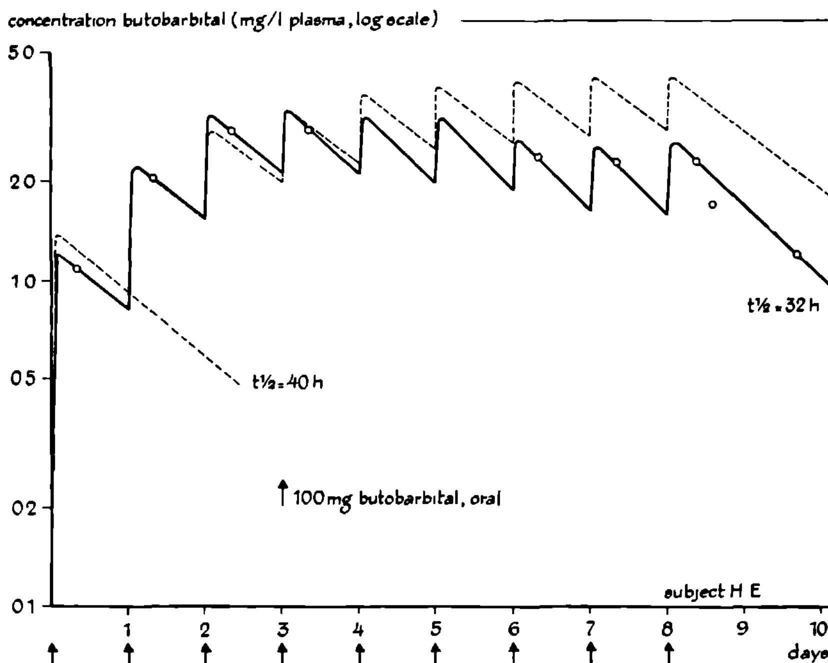

FIG. 4. Simulation of the plasma level profile during repetitive butobarbital administration (solid line). Butobarbital was taken each night and usually 9 h later the plasma concentration was measured. After the last dose the concentration was measured for three more days, in order to determine the half-life of the drug after repetitive dosing. Note the decrease in half-life from 40 h - 32 h, which is probably due to the induction of drug metabolizing enzymes. With the simulation it has been assumed that a gradual decrease in half-life from 40 h to 32 h occurred The dotted line represents the theoretical plasma level profile, based upon the single dose experiment in this subject, with constant half-life. To the concentration at the end of each dosage interval a value equal tot ½A was added in order to obtain the curve for the next interval. Note that accumulation in reality occurred to a lesser extent than theoretically expected. The main reason for this observation is the shortening of the butobarbital half-life during chronic administration.

fact that both drugs have almost equal lipophilic properties (Chapter 1, Section II). The clearance constant is lower than in the case of vinylbital, which thus explains the longer half-life of butobarbital. Elimination occurs largely by means of biodegradation and an important metabolite is 5-ethyl-5-(3-hydroxy-n-butyl)-barbituric acid (Maynert, 1952). This pen-ultimate oxidation is a common pathway in barbiturates with a straight alkyl-chain (Maynert, 1965).

The results after repetitive butobarbital administration are summarized in

TABLE III. Plasma concentrations of butobarbital during and after repetitive oral butobarbital administration*

Subject	day	1	2	3	4	5	6	7	8	9	10
H.E.	dose (mg)	100	100	100	100	100	100	100	100	100	–
	conc. (mg/l)	1.08	2.02	2.85	2.85	n.d.	n.d.	2.35	2.25	2.32	1.42
K.S.	dose (mg)	100	100	100	50	50	50	50	50	50	–
	conc. (mg/l)	1.47	1.89	2.71	2.12	n.d.	n.d.	1.31	1.43	1.40	0.69
F.H.	dose (mg)	200	200	200	–	–	–	–	–	–	–
	conc. (mg/l)	n.d.	n.d.	6.50	3.58	1.90	n.d.	0.55	n.d.	n.d.	0.11
R.C.	dose (mg)	200	200	200	–	–	–	–	–	–	–
	conc. (mg/l)	n.d.	n.d.	6.43	3.10	2.00	n.d.	0.55	n.d.	n.d.	0.09

* Butobarbital was taken usually at midnight and the plasma samples were obtained 9 h later.
n.d. = not determined.

Table III. Accumulation of the drug was evident in the two volunteers who received 200 mg dosages for three nights. The plasma concentration 9 h after the third dose was 6.4 and 6.5 mg/l, which was considerably higher than the average concentration found 9 h after a single dose of 200 mg (3.1 mg/l). The butobarbital half-life had changed in this three-day period from 37 to 30 h (F.H.) and from 40 to 31 h (R.C.). The induction of drug metabolizing enzymes is the most likely explanation for this finding. Similar observations were found for the two volunteers who received 100 or 50 mg of the drug for nine consecutive nights. Accumulation became apparent, although to a lesser extent than expected on basis of the initial half-life of 40 h (Fig. 4; H.E.). The half-life, in this instance, had decreased to 32 h. Volunteer K.S. complained of severe drowsiness during the day and therefore the dose was changed from 100 mg to 50 mg after three nights. The half-life of butobarbital decreased from 35 h before, to 27 h after the chronic experiment.

DISCUSSION

The half-life of butobarbital must be regarded as very long with relation to its intended duration of action in hypnotic drug therapy. During a night's rest of 9 hours no more than 15 - 20% of the administered dose is eliminated. The remaining drug possesses the potentiality of exerting a persistent CNS depressant action during the day. This is obviously undesirable, when no day-time sedation is wanted. In a study on the residual effects of hypnotic drugs, Bond and Lader (1972) tested volunteers with a large number of psychological tests, 12 h after they had received butobarbital (sodium salt, 100 or 200 mg), nitrazepam (5 or 10 mg) or placebo. The psychological tests were interpreted as showing dose-related impairment with both drugs, whereas after butobarbital administration more subjective hang-over was experienced. The observations are consistent with the long persistence of butobarbital in the body and also with the relatively long half-life of nitrazepam, which is 21 - 28 h in man ofter oral administration (Rieder, 1973). The volunteers who participated in the present single dose experiments slept for a few hours after drug intake and complained of sleepiness during the rest of the day. In the case of chronic butobarbital use the situation becomes worse, since the drug accumulates in the body (Fig. 4). The volunteers generally experienced drowsiness the next morning, especially those who had taken 200 mg the previous night. Accumulation on the other hand is counteracted by the induction of metabolic enzyme activity, which becomes evident. The development of this so-called „drug disposition tolerance" (Sharp-

less, 1970) results in a shorter half-life of the drug and is a common phenomenon of barbiturates (Remmer, 1972). The decrease in half-life was about 20 - 25% in the present experiments. A half-life of 14 h was measured in a patient, chronically treated with several psychofarmaca in a neurose-clinic, who received in addition 100 mg butobarbital daily. A similar short half-life of 12 h was reported by Prescott et al. (1973) in a barbiturate addict, following overdosage of the drug. From these observations it follows that the induction of drug metabolizing enzymes may result eventually in a relatively short butobarbital half-life. Also it should be taken into consideration that the enzyme-induction may lead to increased elimination rates of other drugs which are concomitantly administered, e.g. anticoagulants (van Dam, 1968). Whether pharmacodynamic tolerance develops, which involves adaptation of the central nervous system to the presence of the drug, cannot be explained at present. However, it was noticed after interrogating the volunteers, that the increase in plasma concentration during chronic butobarbital administration was not accompanied by a parallel increase in CNS depression. This may indicate that pharmacodynamic tolerance evolves quite readily.

Another consequence of the persistence of butobarbital in the body is the interaction with alcohol, which may lead to dangerous situations. Doenicke (1962) studied the influence of a single dose of 200 mg butabarbital (isomer of butobarbital with an iso-butylgroup instead of the n-butylgroup) combined with alcohol on traffic safety. He concluded that abstention from alcohol is essential for up to 24 h after barbiturate administration, since even small quantities of alcohol in the presence of butabarbital caused severe impairment of physical and mental performance.

CONCLUSIONS

From the pharmacokinetic point of view, butobarbital cannot be regarded as a suitable drug for the treatment of insomnia. Impairment of mental and physical performance during the day is likely to occur, since the half-life of the drug is in the range of 34 - 42 h after a single oral dose. Accumulation takes place when the compound is administered each night, whereas the induction of metabolizing enzymes develops within a few days. This results in a shortening of the half-life of butobarbital itself and possibly also of concomitantly administered drugs. In fact, repetitive butobarbital administration should be avoided and incidental use should be restricted to hospitalized patients, when day-time sedation is wanted, e.g. for preoperative sedation.

SUMMARY

The pharmacokinetics of butobarbital after oral administration were studied. The drug (200 mg) was administered in hard gelatine capsules to five healthy volunteers. Butobarbital plasma concentrations were determined at regular intervals.

Absorption and elimination of butobarbital could be described by single first-order processes. The plasma concentrations were fitted to the equation valid for the one-compartment model after oral administration. The average lag time was 0.11 h and the absorption half-life 0.22 h. The elimination half-life varied from 33.6 to 41.5 h, with an average value of 37.5 h. By assuming that absorption had been complete, an average volume of distribution of 0.78 l/kg and an average clearance constant of 0.24 (ml/min)/kg was estimated. Due to the long half-life of the drug, substantial accumulation of butobarbital occurred during a period of repetitive drug administration. The elimination half-life after these experiments had decreased with about 20 - 25% of its initial value, probably due to the development of enzyme-induction.

It was concluded that butobarbital cannot be regarded as a suitable drug for the treatment of insomnia; repetitive butobarbital administration should be avoided and incidental use should be restricted to patients who require day-time sedation.

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CYCLOBARBITAL CALCIUM ORAL ADMINISTRATION

INTRODUCTION

Cyclobarbital, which was introduced in 1928, is one of the oldest barbiturates used in drug therapy. It has been evaluated in several trials and it was shown to be effective in the treatment of insomnia (e.g., Rushbrooke et al., 1956; Fastier, 1957; Parsons and Thomson, 1961; Itil, 1962; Janke and Schmatzer, 1962; Hinton, 1963). The compound has been classified as an intermediate-acting barbiturate (Martindale, 1972). Since Ahlander (1956) found that cyclobarbital free acid gradually decomposes on storage, the more stable cyclobarbital calcium is now usually preferred. This salt is also available under the trade name PHANODORM (tablets containing 200 mg cyclobarbital calcium). The usual oral dose is 200 - 400 mg at night.

With respect to the pharmacokinetics of cyclobarbital in man, only half-lives after intake of toxic doses have been reported (Brilmayer and Loennecken, 1962). An average elimination half-life of 18 h was found under these conditions. In the present investigation the rate of absorption and elimination in man after oral administration of 300 mg cyclobarbital calcium was estimated. Three preparations, including two types of tablets and an aqueous solution were studied in a cross-over design. This allowed a comparison to be made of the absorption rate and relative bioavailability of the three preparations in the same individual.

METHODS

The following preparations were used:

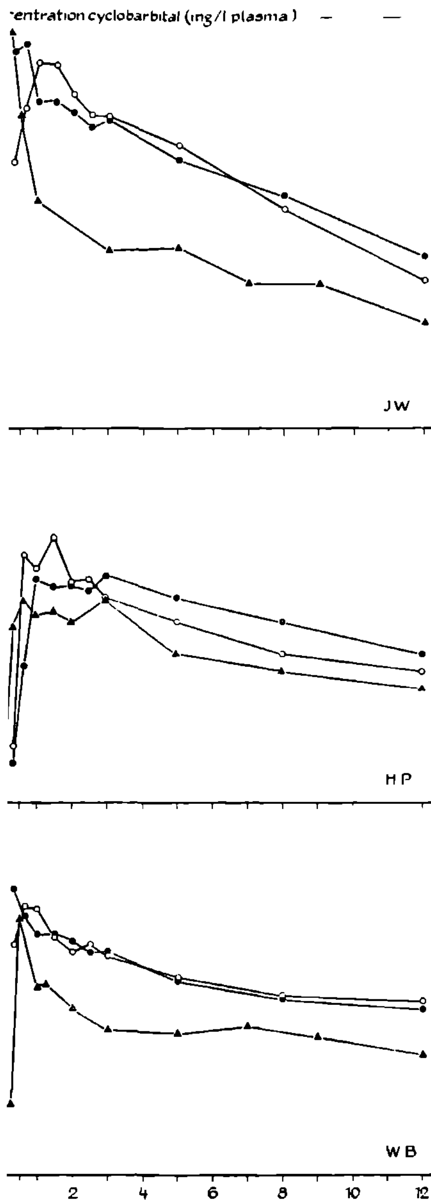
1. F.N.A.-tablets, containing 200 mg cyclobarbital calcium, 60 mg potato starch, 27 mg lactose, 7 mg talc, 3 mg magnesium stearate and gelatine as a granulating agent (*Formularium Nederlandse Apothekers,* 1968). The tablets were prepared at the Dutch Pharmacist's Laboratory, The Hague; the cyclobarbital calcium content was determined in the same laboratory by potentiometric titration with acid (0.1N) in a nitrogen atmosphere. An average content of 104% was found.

2. PHANODORM tablets, containing 200 mg cyclobarbital calcium, obtained from MERCK/BAYER. A cyclobarbital calcium content of 101% was determined.
3. An aqueous solution of cyclobarbital calcium, which was freshly prepared before use (300 mg/150 ml water). The drug was obtained as a bulk substance from Onderlinge Pharmaceutische Groothandel, Utrecht, through the Pharmacy of the St. Radboudhospital, Nijmegen.

Six healthy male volunteers, ranging in age from 21 - 26 years and in bodyweight from 63 - 83 kg, participated in the study. They had received no regular medication during the 4 weeks preceding initiation of the experiments. After an overnight fast, at 9 a.m., the volunteers were given 300 mg cyclobarbital calcium incorporated in one of the above mentioned preparations, together with 150 ml water. They were requested to remain in an upright position for 15 min and then to lie down for at least 3.5 h. This procedure was undertaken in order to simulate as closely as possible the situation of taking a hypnotic drug and going to bed. No food, fluid or tobacco was allowed for at least 3.5 h after drug administration. Blood samples (5 ml) were taken from a forearm vein, usually at he following times: 20 and 40 min., 1, 1.5, 2, 2.5, 3, 5, 8, 12, 24 and 32 h after drug administration. Every volunteer received the three preparations, in a random sequence, with an interval of at least 1 week. Cyclobarbital concentrations in plasma were determined by gas chromatography, as described in Chapter 8, Section II.

RESULTS

In Fig. 1 all of the individual plasma concentration curves are shown, which were obtained after oral administration of the three preparations to each volunteer. In most instances the cyclobarbital plasma concentration rose rapidly, suggesting a rapid absorption of cyclobarbital when administered as the calcium salt. In Table I the peak level times (t_{max}) are given. Slight differences between the preparations became apparent for almost every volunteer. The most rapid absorption occurred when the drug was administered in aqueous solution; generally the peak concentrations were attained within one hour after administration. PHANODORM and the F.N.A.-tablets may be regarded as equivalent with respect to their absorption rate. The maximum concentrations reached showed little variation among the three preparations. Drowsiness was experienced by the volunteers, usually 15 - 30 min after intake of each preparation. Cyclobarbital plasma concentrations were approximately 3 mg/l or higher when feelings of CNS depression became apparent. The volunteers were inclined to sleep for three or four hours before they were able to go for lunch.



ı. 1. Plasma concentration curves of c
mg cyclobarbital calcium as F.N.A.-tab
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TABLE I. Absorption characteristics of three cyclobarbital calcium preparations after oral administration to the same healthy volunteers.

Subject	Body-weight (kg)	Dose (mg)	Dose/kg (mg)	Preparation	t_{max} (min)	C_{max} (mg/l)
H.P.	70	300	4.29	F.N.A.	120	8.5
				PHANODORM	90	8.5
				Solution	40	5.8
R.D.	72	300	4.17	F.N.A.	60	7.7
				PHANODORM	60	8.3
				Solution	60	8.8
J.W.	63	300	4.76	F.N.A.	40	10.8
				PHANODORM	60	10.3
				Solution	15	11.7
B.W.	73	300	4.19	F.N.A.	20	9.8
				PHANODORM	40	9.8
				Solution	20	9.7
W.B.	83	300	3.61	F.N.A.	20	8.1
				PHANODORM	40	7.6
				Solution	30	7.2
G.W.	72	300	4.17	F.N.A.	180	7.3
				PHANODORM	150	7.4
				Solution	90	6.6
Mean values	72	300	4.17	F.N.A.	73	8.3
				PHANODORM	73	8.7
				Solution	42	8.3

Occasionally, they reported that drowsiness was absent by this time, while at other times they reported persistent lethargy for the remainder of the afternoon.

Although absorption generally occurred quite regularly, it was not possible to deduce the correct absorption parameters according to single first-order kinetics from each concentration curve. This was mostly due to the lack of sufficient data points during the absorption phase. With early peak concentrations an initial rapid decline of the plasma concentration was often observed.

TABLE II. Elimination half-lives and relative bioavailability of cyclobarbital after oral administration of three cyclobarbital calcium preparations to the same healthy volunteers.

Subject	Preparation	$t_{1/2}$ (h)	average τ_{el} (h)	AUC 0-12 h (mg.h/l)	conc. 12 h (mg/l)	AUC 0 - ∞ (mg.h/l)	F_{rel} (%)
H.P.	F.N.A.	17		60.9	4.2	156.7	100
	PHANODORM	15.5		67.5	3.7	151.9	97
	Solution	15		49.9	3.2	122.9	78
		$\overline{15.8}$	22.8				
R.D.	F.N.A.	10		55.0	3.0	99.6	100
	PHANODORM	11		70.0	4.4	135.5	136
	Solution	10		59.6	2.7	99.8	100
		$\overline{10.3}$	14.9				
J.W.	F.N.A.	11		85.2	4.9	155.8	100
	PHANODORM	10		84.3	4.2	144.8	93
	Solution	9		56.9	3.0	100.1	64
		$\overline{10}$	14.4				
B.W.	F.N.A.	9.5		62.7	3.3	105.5	100
	PHANODORM	9.5		58.6	3.3	101.4	96
	Solution	8		57.0	2.8	93.3	88
		$\overline{9}$	13.0				
W.B.	F.N.A.	17		66.1	4.7	176.6	100
	PHANODORM	17		65.2	4.8	180.5	102
	Solution	15		49.1	3.4	129.1	73
		$\overline{16.3}$	23.5				
G.W.	F.N.A.	8		67.2	3.9	115.9	100
	PHANODORM	9		60.8	3.4	103.2	89
	Solution	9		51.9	2.8	86.8	75
		$\overline{8.7}$	12.5				
Mean values							
	F.N.A.	12.1		66.2	4.0	135.0	100
	PHANODORM	12.0		67.7	4.0	136.2	101
	Solution	10.7		54.1	3.0	105.3	78
		$\overline{11.6}$	16.7				

This suggests that, in such a situation, distribution of the drug from plasma into tissues is slower than drug absorption. Similar observations were encountered for hexobarbital sodium and heptabarbital sodium (Chapter 1 and 5, Section III). The subsequent slower decrease of plasma concentration represents the elimination phase, which proceeds according to a single first-order process. On all occasions straight lines were obtained for the decrease in plasma concentration after plotting on semi-logarithmic scale (Fig. 2). The plasma half-lives of cyclobarbital varied from 8 - 17 h (Table II). In addition, it was observed that the half-lives of the barbiturate were about the same for each individual on the three occasions (Fig. 2; Table II). The small differences measured may not be meaningful, since they are in the same order as the experimental error.

With respect to relative bioavailability estimation of cyclobarbital, the same procedure was applied as described in detail for heptabarbital (Chapter 5, Section III). It was assumed that the total body clearance was constant for a certain individual. The areas under the plasma concentration curves were estimated, including a correction for the undetermined area. The bioavailability was calculated relative to the F.N.A.-tablets (Table II). Surprisingly, the aqueous

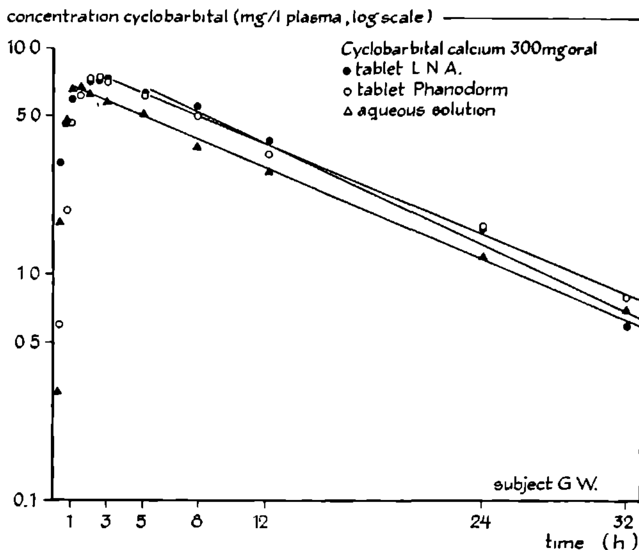

FIG. 2. Plasma concentration curves of cyclobarbital plotted on semi-logarithmic scale, representing the elimination phase of cyclobarbital after oral administration of the three preparations to subject G.W.; F.N.A.-tablets (solid circles), PHANODORM (open circles) and aqueous solution (triangles). The elimination half-life of cyclobarbital in this subject varied from 8 to 9 h.

solution showed the lowest bioavailability. Absorption of cyclobarbital from the F.N.A.-tablets and from PHANODORM generally occurred to a comparable extent.

If the absolute bioavailability of cyclobarbital for the F.N.A.-tablets is assumed to be 100%, then the average clearance constant and apparent volume of distribution may be estimated. The zero-time concentration (A) of the average plasma concentration curve for the F.N.A.-tablets was found to be 8 mg/l. Since absorption is much more rapid than elimination, an estimation for V_f may be obtained by the quotient Dose/A, whereas $k_{el} = V_f/1.44. t_{1/2}$. This yields 37 l for V_f and 35 ml/min for k_{el} of cyclobarbital. These values may be regarded as relatively low when compared with the other barbiturates studied. The small apparent volume of distribution is in accordance with its relatively low lipophility (Chapter 1, Section II).

Cyclobarbital, for the major part, is eliminated by metabolism. In 1932 Fretwurst et al. reported that only 2.5 - 6.3% of the administered dose was excreted as unchanged drug in the urine. More recently some oxidized metabolites were detected in human urine after cyclobarbital administration (Seifert, 1956; Frey et al., 1959; Eberhardt et al., 1962). These were identified by Arnold and Grützmacher (1969) as 3'-keto- and 3'-hydroxy-cyclobarbital, with the aid of mass spectrometry.

DISCUSSION

The complete cross-over design in this investigation on the kinetics of cyclobarbital calcium in man, permits a comparison to be made of the absorption and elimination parameters in the same volunteer. Each individual serves as his own control, which minimizes the variability attributable to physiological factors. Differences in the absorption rate or bioavailability of a drug from certain dosage forms, encountered in the same volunteer, may now be attributed to pharmaceutical factors rather than to physiological factors. Although there is a substantial variation between individuals, it is evident that the rank order in absorption rate and bioavailability of the three cyclobarbital calcium containing preparations is generally the same for each volunteer. For instance, the absorption of cyclobarbital was relatively slow for subject G.W., but still the solution was absorbed more rapidly and also to a lesser extent.

The calcium salt of cyclobarbital is appreciably soluble in water (1 g/100 ml; Martindale, 1972) which explains the rapid absorption of the preparations used in this study. The importance of salt formation and aqueous solubility for barbiturate absorption was discussed in detail for heptabarbital (Chapter 5,

Section III). As expected, the cyclobarbital calcium solution showed the highest absorption rate, since the primary dissolution of the active ingredient, which often is a rate determining step in drug absorption (Benet, 1973), does not apply in the case of an aqueous solution. However, the finding that the solution showed the lowest bioavailability in these experiments, is unexpected. Often it is recommended in comparative bioavailability studies to use an aqueous solution of a water-soluble drug as the reference dosage form (*Guidelines,* 1972). This is rational, since it eliminates all of the factors involved in the dissolution process. Such factors are often involved when bioavailability is found to be reduced (Wagner, 1971). With barbiturate salts a complicating factor may be the fact that the poor solubility of the free acid causes precipitation when the drug reaches the acidic gastric fluids. The precipitate which is formed after cyclobarbital calcium solution administration, may have unfavourable redissolution properties when compared with the precipitate formed after tablet administration. However, this only counts for part of the precipitate, since the rapid absorption of the solution also suggests that rapid dissolution occurs. Additional experiments, in vitro and in vivo, are required in order to determine the cause of the lower bioavailability of cyclobarbital from the solution.

The half-life of cyclobarbital, for four of the volunteers who participated in the present experiments, were between 8 and 11 h. This is relatively short and advantageous with respect to the intended duration of action in the treatment of insomnia. After a night's rest of 9 h approximately 40 - 55% of the drug has been inactivated and it is likely that the plasma concentration has decreased below the minimal effective level, if a normal dose of 200 or 300 mg has been given. This level varied from 3 - 7 mg/l with respect to the onset of action. The duration of action, as judged by subjective feelings of CNS depression, varied considerably among the volunteers. Janke and Schmatzer (1962) observed impairment of mental performance in volunteers 12 h after they had received 400 mg cyclobarbital calcium. Obviously, the risk of residual effects after a lower dose is less, whereas it may still be effective in the induction and maintenance of sleep. On the other hand, also the longer half-life of cyclobarbital in the two volunteers should be considered. This was 15 - 17 h, which must be regarded as too long for rational hypnotic drug treatment. Residual effects may be expected the following morning. Only when day-time sedation is required, should such a long half-life be advantageous. The reason for the considerable difference in half-life of cyclobarbital between the subjects is not clear. Many factors may be involved, which were discussed recently by Gillette (1971). At present it is difficult to judge whether cyclobarbital could be safely used in a certain individual for the treatment of insomnia, since a relatively

long half-life should be reckoned with. Furthermore, experiments of repetitive cyclobarbital calcium administration are required, in order to ascertain if important accumulation of the drug in the body becomes apparent and also to determine if the half-life of the drug remains unchanged. A preliminary experiment has revealed that substantial accumulation did not occur and that the half-life had not changed after repetitive cyclobarbital calcium administration (200 mg every night).

CONCLUSIONS

These pharmacokinetic studies reveal that the half-life of cyclobarbital in some subjects is sufficiently short to be regarded as suitable for the treatment of insomnia. Due to the fact that a much longer half-life became apparent in some other subjects, cyclobarbital cannot, however, uniformly be recommended for use in general practice. For subjects with a half-life of 15 - 17 h residual effects are likely to occur the following morning.

In principle, the calcium salt containing preparations are suitable for the rapid induction of sleep, since the rate of absorption of cyclobarbital from these preparations is rapid.

SUMMARY

The pharmacokinetics and relative bioavailabililty of cyclobarbital calcium were studied after oral administration. Tablets, according to the Formularium Nederlandse Apothekers (1968), PHANODORM, and an aqueous solution were used. Six healthy volunteers participated on three occassions in this investigation and received the three preparations. The administered dose was 300 mg cyclobarbital calcium. Cyclobarbital plasma concentrations were determined at regular intervals.

The absorption of cyclobarbital from the three preparations was rapid and highest for the solution. Peak concentrations were usually attained within 1 h. The elimination of cyclobarbital could be described by a single first-order process with an average half-life of 11.6 h (range 8 - 17 h). Little intrasubject variation was noted with respect to the half-life. Relative bioavailability was estimated for each volunteer by comparing the areas under the plasma concentration curves. The F.N.A.-tablets and PHANODORM showed comparable bioavailability, whereas for the solution an average bioavailability of 78% relative to the F.N.A.-tablets was estimated; the reason for this unexpected finding is unknown.

It has been concluded that cyclobarbital cannot uniformly be regarded as a suitable drug for the treatment of insomnia. The long half-life that became apparent in some of the volunteers (15 - 17 h) causes a substantial risk of residual effects to occur the following morning. In principle, the calcium salt of cyclobarbital may be used for rapid sleep induction, because of its rapid absorption.

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CHAPTER 8

CHLORAL HYDRATE

A. GENERAL INTRODUCTION

B. PHARMACOKINETICS AND METABOLISM

C. BIOPHARMACEUTICS

1. RECTAL ADMINISTRATION
2. ORAL ADMINISTRATION

A. GENERAL INTRODUCTION

Chloral hydrate ($CCl_3CH(OH)_2$) was first synthesized by Liebig in 1832. Liebreich (1869) described its hypnotic action and he suggested that the drug was effective because it was metabolized in the body to chloroform. However, subsequent work failed to substantiate this hypothesis (Tomaszewicz, 1874). Von Mering (1882) found that the product isolated from the urine of patients receiving chloral hydrate was a conjugation product of trichloroethanol and glucuronic acid. Külz (1882, 1884) showed that trichloroethanol has hypnotic activity and he proposed that the pharmacological effect of chloral hydrate may be attributed to trichloroethanol, which is formed in the body by reduction of the parent drug. This theory has been supported by the work of Butler (1948) and of Marshall and Owens (1954), who found that the reduction of chloral hydrate to trichloroethanol occurs so rapidly in man and in dogs, that it is difficult to detect any unchanged chloral hydrate in the blood or plasma after oral administration. In comparative evaluations of the clinical efficacy of the two compounds, trichloroethanol was found to be at least as effective as chloral hydrate (Owens et al., 1955; Imboden and Lasagna, 1956). On the other hand Mackay and Cooper (1962) as well as Buchel (1964) have shown that chloral hydrate itself has hypnotic activity. In a recent study, Grüner et al. (1973) compared the effects of chloral hydrate and of trichloroethanol on the EEG of

the isolated perfused rat brain and they found that both compounds exhibited CNS depressant activity.

In conclusion, it seems most likely that chloral hydrate has in itself hypnotic properties, its action being limited, however, by a rapid reduction to trichloroethanol and the latter compound continuing the hypnotic activity. Only after rapid i.v. administration, chloral hydrate must be expected to contribute substantially to the induction of sleep. After oral or rectal administration the sedative or hypnotic action must be attributed entirely to trichloroethanol, since no measurable amount of parent drug reaches the general circulation intact (Marshall and Owens, 1954; Breimer, Ketelaars and van Rossum, unpublished results). Thus, chloral hydrate may be regarded as a transport form for the active metabolite trichloroethanol (Breimer, 1973).

Marshall and Owens (1954) showed that, with respect to absorption and excretion rates, it probably did not make a great difference whether chloral hydrate or trichloroethanol was given. One advantage of using chloral hydrate, in general practice, is the fact that it is a solid, whereas trichloroethanol is a liquid which is more difficult to handle. Both compounds are appreciably soluble in water, while trichloroethanol also has substantial lipophilic properties (Garrett and Lambert, 1966). It is not known whether the side-effects of chloral hydrate such as irritation to mucous membranes and occasional nausea and vomiting, are different with trichloroethanol. Usually it is recommended that chloral hydrate should be administered as a dilute aqueous solution in order to prevent these side-effects. In a recent article Fairbrother (1973) has reviewed extensively the physical and chemical properties of chloral hydrate.

Chloral hydrate was introduced into medicine when only alcohol, opium and Cannabis were available for hypnosis and sedation. Although its popularity, in the treatment of insomnia, diminished after the introduction of the barbiturates, it is still a widely used hypnotic drug, especially in children and in elderly patients (Ferguson Anderson, 1973). Also in the treatment of infantile convulsions the drug seems to be of value (Renner, 1962; Martindale, 1972). The central depressant actions of chloral hydrate, or better still of trichloroethanol, resemble those of alcohol, the barbiturates and the gaseous anaesthetics. Like many hypnotic drugs, chronic use of chloral hydrate can lead eventually to habituation, tolerance and addiction (Maynert, 1965). Small doses produce sedation (0.5 g), larger doses facilitate sleep (1 - 2 g), whereas overdosage leads to anaesthesia or coma (4 - 20 g). Clinical trials have proved the effectiveness of chloral hydrate (the usual hypnotic doses of 1 or 2 g) in inducing and maintaining sleep in adults (Owens et al., 1955; Imboden and Lasagna, 1956; Shapiro et al., 1969). Rickels and Bass (1963) however, showed that the dose of 1 g was only slightly better than a placebo and it seems likely that many individuals

may require 1.5 or 2 g. Up to 200 mg may be given to infants under 1 year of age, 250 - 500 mg to children from 1 to 5 years and 500 - 1000 mg from 6 to 12 years (Martindale, 1972). For preoperative sedation 40 - 60 mg/kg can be used (Anderson, 1962; Illingworth, 1968).

Chloral hydrate may be advantageous when compared with the barbiturates, since Kales et al. (1969) reported that chloral hydrate, as well as certain benzodiazepines, did not suppress REM-sleep. However chloral hydrate was administered, in these experiments, in doses of 0.5 and 1.0 g, which can hardly be considered to be effective hypnotic doses (Sharpless, 1970). Whether larger doses would suppress REM-sleep remains unknown.

From the pharmacokinetic and biopharmaceutical point of view, no satisfactory literature data are available to judge the usefulness of chloral hydrate in rational hypnotic drug therapy. It was for this reason that the present investigations were initiated, as will be further outlined in the following. Besides, the assay method which was developed (Chapter 9, Section II; Breimer et al., 1974) not only permitted the blood and urine analysis of the active compound trichloroethanol, but also of some other metabolites of chloral hydrate. Therefore, the kinetics of these compounds were studied simultaneously.

B. PHARMACOKINETICS AND METABOLISM

INTRODUCTION

As already mentioned chloral hydrate is very rapidly reduced to trichloroethanol; this reaction occurs in the liver and in other tissues, including erythrocytes (Butler, 1949; Sellers, Lang et al., 1972). Friedman and Cooper (1960) found that the reduction is catalyzed by the enzyme alcohol dehydrogenase. Due to this observation, quite some attention has been paid to the interaction between chloral hydrate and alcohol. Concurrent administration enhances the depressant action of chloral hydrate (Kaplan et al., 1969; Gessner and Cabana, 1970; Sellers et al., 1972a). Kaplan et al. (1967) and Sellers et al. (1972) showed that the interaction in man results in higher trichloroethanol and also in higher alcohol blood concentrations.

A small amount of trichloroethanol is excreted unchanged into the urine, the rest is further metabolized by conjugation with glucuronic acid (urochloralic acid) before excretion (Marshall and Owens, 1954).

Apart from reduction, chloral hydrate can be oxidized to trichloroacetic acid

(Butler, 1948; Marshall and Owens, 1954). This conversion is catalyzed by NAD-dependent aldehyde dehydrogenase in the liver, for which, surprisingly, no naturally occurring substrate could be found (Cooper and Friedman, 1958). Purified human acetaldehyde dehydrogenase does not metabolize chloral hydrate (Kraemer and Deitrich, 1968; Blair and Bodley, 1969). The enzyme which catalyzes the oxidation is not yet known. Also, after trichloroethanol administration trichloroacetic acid has been detected (Marshall and Owens, 1954). Is seems likely that in this case chloral hydrate is an intermediate, but in in vitro studies this compound could not be identified (Friedman and Cooper, 1960). Trichloroacetic acid is predominantly excreted unchanged into the urine (Paykoç and Powell, 1954).

In Fig. 1 the fate of chloral hydrate in the body is summarized, as far as known up to now. Few quantitative studies on the pharmacokinetics of chloral

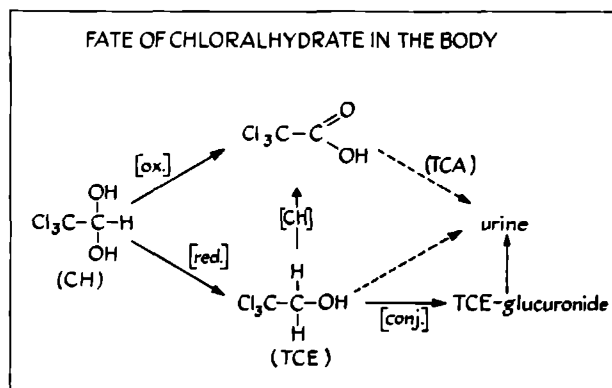

FIG. 1. Schematic representation of the fate of chloral hydrate in man.

hydrate and its metabolites in man have been carried out. The investigations by Marshall and Owens (1954) and Owens and Marshall (1955) give detailed information, however, their data hardly permit the calculation of half-lives or other important pharmacokinetic parameters. Sellers et al. (1972) determined an average half-life of 8.2 h for trichloroethanol, when studying the interaction of chloral hydrate and alcohol. It should be mentioned further that Garrett and Lambert (1973) extensively investigated the kinetics of trichloroethanol and metabolites in dog.

It was the aim of the present investigation to study the pharmacokinetics of chloral hydrate and its known metabolites in man. This became possible since the assay method permits the simultaneous determination of chloral hydrate,

trichloroethanol, trichloroethanol-glucuronide and trichloroacetic acid in blood and in urine. Chloral hydrate was administered orally and rectally in this study, since both routes of administration are important in general practice. Pharmacokinetic data after rectal chloral hydrate administration seem to be completely lacking in the literature.

METHODS

Oral administration

Six healthy male volunteers, aged 20 - 26 years, who did not take any regular medication, participated in the study. They were not allowed to consume alcoholic beverages from 12 h before the experiment until 24 h after the experiment. The trials began in the morning after the subjects had fasted overnight and 15 mg/kg of chloral hydrate (obtained from OPG, Utrecht), dissolved in 150 ml water, was administered orally. One volunteer (J.G.) received trichloroethanol 15 mg/kg (obtained from Koch & Light, Colnbrook, Great Britain) dissolved in 150 ml water. After intake of the drug the subjects were asked to stay in an upright position for 15 min and then to lie down for at least two hours. Usually blood samples were taken by vein puncture at 10, 20, 30, 40 and 60 min and further at 1.5, 2, 3, 5, 7, 9 and 11 or 12 h after drug intake. The same subjects collected their urine at frequent intervals for about 50 hours. Subject A.H. collected his urine over a period of 9 days.

Total blood concentrations and urine concentrations of trichloroethanol, trichloroethanol-glucuronide and trichloroacetic acid were determined by gas chromatography with electron capture detection, utilizing head-space analysis, as described in Chapter 9 of Section II. In subject J.G. there was no differentiation made between free and conjugated trichloroethanol.

Subject J.H. received chloral hydrate also for three consecutive nights after the start of the trial and for several days the trichloroacetic acid blood concentration was determined.

Rectal administration

For three volunteers, who participated in the study of the biopharmaceutical aspects of rectal chloral hydrate administration, also the concentration of trichloroethanol-glucuronide and of trichloroacetic acid in blood was followed. Two subjects received the drug (1000 mg), dissolved in polyethylene glycol 300 (PEG 300) and another received it dissolved in sesame oil. A rectiole was used for the introduction of the solutions into the rectum. Details concerning the

preparation and the composition of these dosage forms are given in this chapter under "biopharmaceutics of rectal chloral hydrate administration". For the rest the procedure as described under "oral administration" was followed.

RESULTS

After oral and rectal administration of chloral hydrate no unchanged parent drug could be detected in the blood. There was a rapid rise of trichloroethanol concentration after oral intake of the aqueous solution. Peak levels were attained after 20 - 60 min with a mean height of 7.6 mg/l (range 5.2 - 11.2 mg/l). In most subjects the trichloroethanol-glucuronide concentration reached its maximum value at the same time as the free trichloroethanol. In Table I these results have been summarized. After the absorption had finished a rapid decline in trichloroethanol concentration occurred, followed by a slower monoexponential decay. A similar profile was found for trichloroethanol-glucuronide. In Fig. 2 a typical example of the blood concentration - time curves is shown as measured for subject A.H. In Table II the blood half-lives of trichloroethanol and of trichloroethanol-glucuronide are given, together with the trichloroacetic acid concentrations at 12 h. The average half-life of the slower phase was 8.0 h for trichloroethanol (range 6.5 - 9.5 h) and 6.7 h for trichloroethanol-glucuronide (range 6.0 - 8.0 h). Also trichloroacetic acid was rapidly formed after a single oral dose of chloral hydrate. A relatively high concentration of this

TABLE I. Peak blood concentrations of trichloroethanol and trichloroethanol-glucuronide after oral administration of chloral hydrate in aqueous solution to five human volunteers.

Subject	Body-weight (kg)	Dose (mg)	Dose/kg (mg)	Trichloro-ethanol C_{max} (mg/l)	Trichloro-ethanol t_{max} (min)	Trichloroethanol-glucuronide C_{max} (mg/l)	Trichloroethanol-glucuronide t_{max} (min)
H.H.	70	1050	15.0	11.2	20	10.8	20
W.E.	76	1140	15.0	7.6	60	2.7	60
T.L.	54	810	15.0	6.5	40	5.7	40
P.W.	65	975	15.0	7.4	60	6.6	30
A.H.	85	1275	15.0	5.2	20	7.9	20
mean	70	1050	15.0	7.6	40	6.7	34

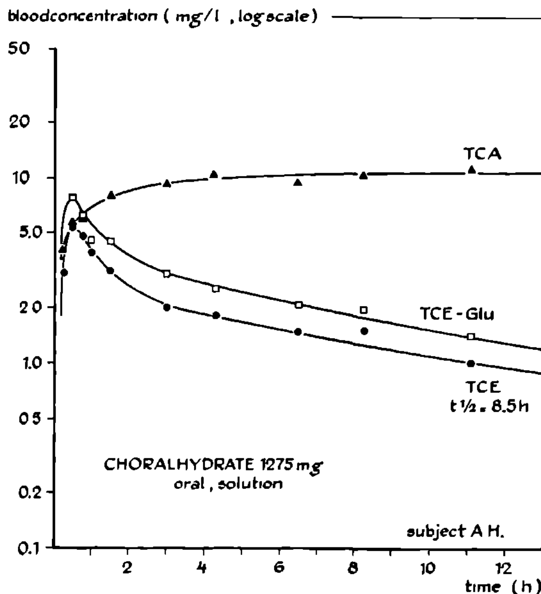

FIG. 2. Blood concentration curves on semi-logarithmic scale of trichloroethanol (TCE), trichloroethanol-glucuronide (TCE-Glu) and trichloroacetic acid (TCA) after oral administration of chloral hydrate (15 mg/kg) in aqueous solution.

metabolite in the blood was reached within one hour. Even to 12 h an increase in this concentration was noticed (Fig. 2). The half-life of trichloroacetic acid appeared to be about 4 days (100 h) in subject A.H. (Fig. 6).

After rectal administration of chloral hydrate the half-lives of the compounds measured were in the same range as when chloral hydrate was given orally (Table III). Also, trichloroacetic acid accumulated for at least 12 h. In Fig. 3 the blood concentration - time curves are shown for subject K.S. Absorption apparently occurred much slower than after oral intake of an aqueous solution. Details of the rectal absorption characteristics and the influence of vehicle composition will be given in this chapter under "biopharmaceutics of rectal chloral hydrate administration".

The results of the one subject receiving trichloroethanol are given in Fig. 4. No differentiation was made between free trichloroethanol and trichloroethanol-glucuronide, since the aim of this single experiment was just to find out whether (also after trichloroethanol administration) a substantial amount of trichloroacetic acid would be formed. This does indeed occur, although its initial rate of formation is slower than after chloral hydrate administration.

TABLE II. Blood half-lives of trichloroethanol and trichloroethanol-glucuronide after oral administration of chloral hydrate. Blood concentrations of trichloroacetic acid at 12 h.

Subject	Trichloroethanol $t_{1/2}$ (h)	Trichloroethanol-glucuronide $t_{1/2}$ (h)	Trichloroacetic acid blood concentration at 12 h (mg/l)
H.H.	8.5	6.5	16.7
W.E.	6.5	6.0	17.4
T.L.	9.5	6.0	10.7
P.W.	7.0	7.0	11.1
A.H.	8.5	8.0	11.0 (11 h)
mean	8.0	6.7	13.4

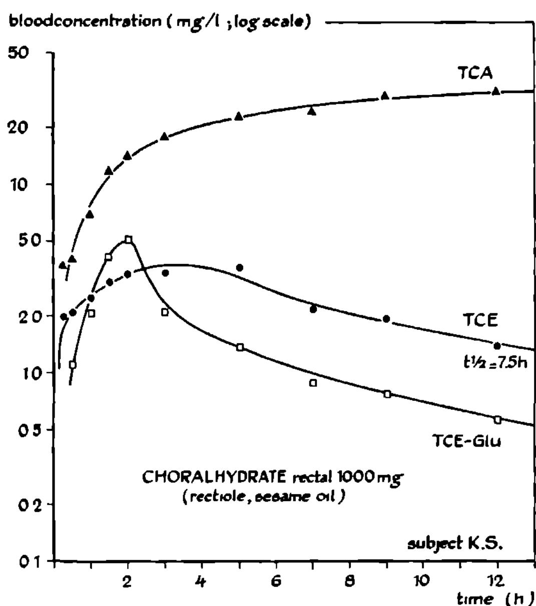

FIG. 3. Blood concentration curves on semi-logarithmic scale of trichloroethanol (TCE), trichloroethanol-glucuronide (TCE-Glu) and trichloroacetic acid (TCA) after rectal administration of chloral hydrate (1000 mg) dissolved in 3 ml sesame oil.

TABLE III. Blood half-lives of trichloroethanol and trichloroethanol-glucuronide after rectal administration of chloral hydrate. Blood concentrations of trichloroacetic acid at 12 h.

Subject	Body-weight (kg)	Dose (mg)	Dose/kg (mg)	Trichloro-ethanol $t_{1/2}$ (h)	Trichloro-ethanol-glucuronide $t_{1/2}$ (h)	Trichloro-acetic acid blood concentration at 12 h (mg/l)
K.S.	67	1000	14.9	7.5	7.0	29.8
P.A.	65	1000	15.2	8.5	8.0	27.3
R. T.	66	1000	15.1	7.0	determination not possible	12.9

For the five subjects receiving chloral hydrate orally, also the urinary excretion of trichloroethanol, trichloroethanol-glucuronide and trichloroacetic acid was determined for about 50 h (Table IV). As expected, no unchanged chloral hydrate could be detected and only a small amount was excreted as free trichloroethanol (2.6 - 7.7 mg). Varying amounts of the glucuronide were found: 108 - 307 mg (calculated as chloral hydrate), which is equivalent to 10 - 24% of the dose administered. The amount of trichloroacetic acid excreted

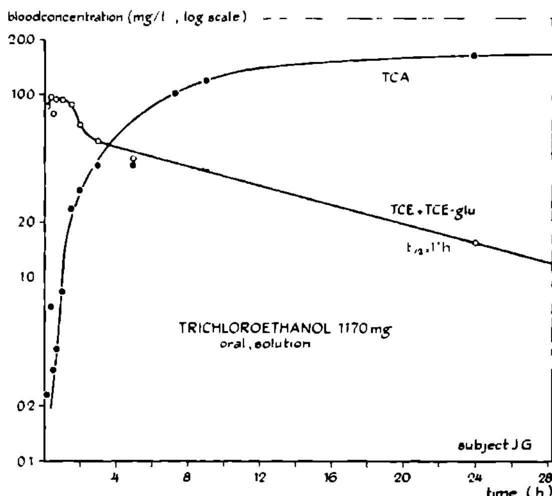

FIG. 4. Blood concentration curves on semi-logarithmic scale of free + conjugated trichloroethanol (TCE + TCE-Glu) and of trichloroacetic acid (TCA) after oral administration of trichloroethanol (15 mg/kg) in aqueous solution.

in the first 50 h is not the total quantity to be excreted, because of the very long half-life of this compound. This can be seen in Fig. 6, where a plateau for cumulative trichloroacetic acid excretion has not been reached even after 9 days. Urine collection during 15 - 20 days will be required to measure all of the trichloroacetic acid formed after a single dose of chloral hydrate.

In general, the rate of renal elimination of a drug is directly proportional to the plasma - water concentration (C_p):

$$\frac{dQ_r}{dt} = k_r \cdot C_p$$

where $\frac{dQ_r}{dt}$ is the renal excretion rate (mg/h) and k_r is the renal clearance constant (ml/min or l/h). The curve, obtained by plotting the renal excretion rate versus time, should have the same shape as the plasma-water concentration curve. This might also be true for the total blood concentration curve, if protein binding is relatively low or dissociation of the drug-protein complex is very rapid and exchange, alternatively, between plasma and erythrocytes is a rapid process or does not occur at all. Protein binding of trichloroethanol and its glucuronide is about 37% (determined at concentrations of 5 mg/l and 9 mg/l respectively), while the former does and the latter does not partition into red blood cells (Garrett and Lambert, 1973). For trichloroacetic acid the situation may be more complicated as its binding to plasma proteins is much higher:

TABLE IV. Cumulative urinary excretion of trichloroethanol, trichloroethanol-glucuronide and trichloroacetic acid after oral administration of chloral hydrate (calculated as chloral hydrate).

Subject	Dose (mg)	Trichloroethanol (mg)	Trichloroethanol-glucuronide (mg)		Trichloroacetic acid (mg)	
H.H.	1050	2.6	239	(23%)[1]	8.7	(48 h)[2]
W.E.	1140	7.7	108	(11%)	55.6	(42 h)
T.L.	810	5.3	147	(19%)	46.2	(32 h)
P.W.	975	4.9	149	(15%)	36.9	(52 h)
A.H.	1275	3.5	307	(24%)	90.1	(216 h)

[1]) Percentage of the dose excreted in 48 h.

[2]) Time interval in hours, after intake of chloral hydrate, during which trichloroacetic acid was measured in urine.

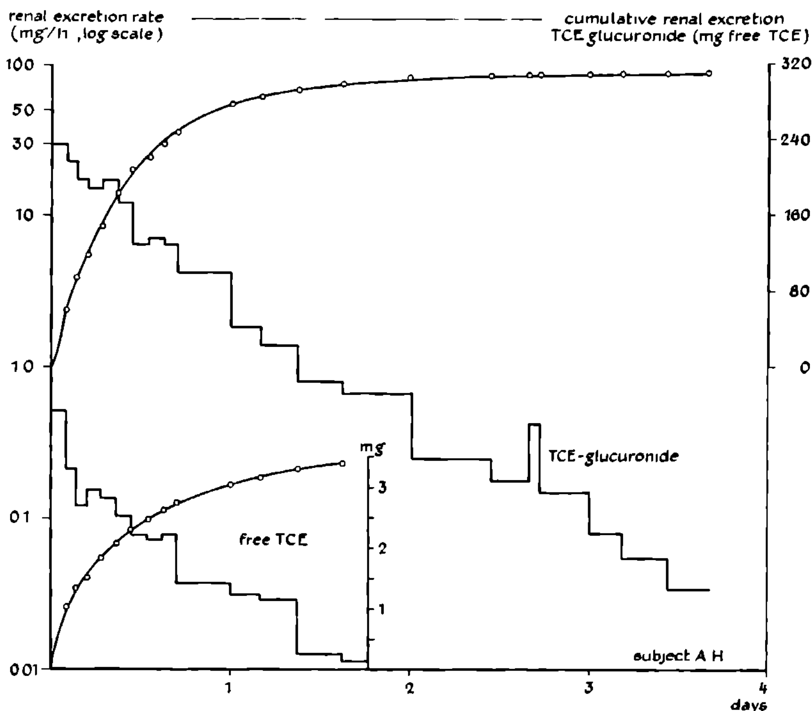

FIG. 5. Average renal excretion rate curves (semi-logarithmic scale) and cumulative renal excretion of trichloroethanol (free TCE) and trichloroethanol-glucuronide (TCE-glucuronide) after oral administration of chloral hydrate (1275 mg). See Fig. 2 for the blood concentration curves of this subject.

84 and 94% determined at plasma concentrations of 100 mg/l and 30 mg/l respectively (Sellers and Koch-Weser, 1971). In cases where the above equation is valid, the biological half-life can be deduced from the renal excretion rate curve. Experimentally the excretion rate is not determined over infinitely small time intervals, but over discrete periods of time (Fig. 5 and 6). Therefore, it is sometimes more convenient to use the renal excretion deficit for the calculation of half-life. If single-compartment kinetics are valid and absorption is fast compared to elimination, the following applies (van Rossum, 1971):

$$\log (Q_{r,\, t\, =\, \infty} - Q_{r,\, t}) \;=\; \log Q_{r,\, t} - 0.301\; t/t_{1/2}$$

where $Q_{r,\, t\, =\, \infty}$ is the total amount excreted (mg), $Q_{r,\, t}$ is the amount excreted until time t (mg) and $(Q_{r,\, t\, -\, \infty} - Q_{r,\, t})$ is the excretion deficit. The log of the

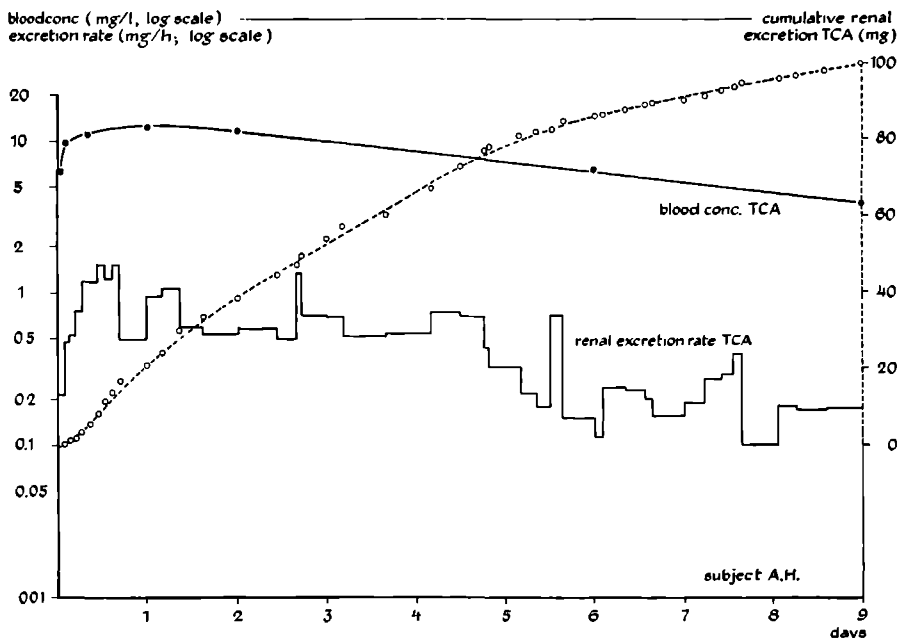

FIG. 6. Average renal excretion rate curve, blood concentration curve (semi-logarithmic scale) and cumulative renal excretion of trichloroacetic acid (TCA) after oral administration of chloral hydrate (1275 mg). These data are from the same experiment as Fig. 2 and Fig. 5. The blood half-life of TCA is about 100 h.

renal excretion deficit is a linear function of time. With trichloroethanol and trichloroethanol-glucuronide indeed straight lines have been obtained, as shown in Fig. 7. In Table V the urine half-lives of the two compounds are given and they appear to be somewhat longer than the blood half-lives.

DISCUSSION

Chloral hydrate

The reduction of chloral hydrate seems to occur more rapidly than chloral hydrate absorption, since no unchanged parent drug can be detected, even in the first blood sample. On the other hand already a substantial concentration of trichloroethanol is measured at this time. As soon as chloral hydrate reaches the blood in the intestinal wall capillaries the reduction by the erythrocytes begins and during the first passage through the liver the remainder is reduced

TABLE V. Half-lives of trichloroethanol and trichloroethanol-glucuronide obtained from urinary excretion deficit curves.

Subject	Trichloroethanol $t_{1/2}$ (h)	Trichloroethanol-glucuronide $t_{1/2}$ (h)
H.H.	—[1]	7.5
W.E.	10.0	8.0
T.L.	10.0	8.0
P. W.	11.5	10.5
A.H.	9.0	7.5
mean	10.1	8.3

[1]) Irregular excretion.

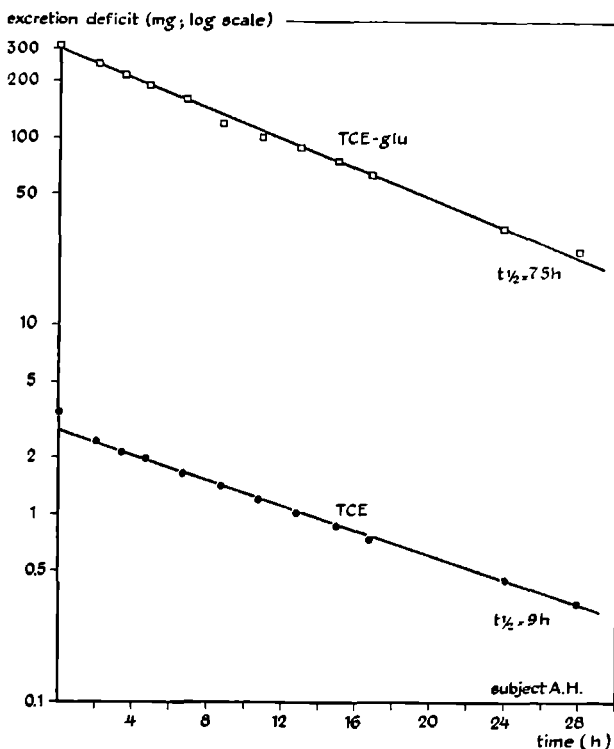

FIG. 7. Renal excretion deficit curves on semi-logarithmic scale of trichloroethanol (TCE) and trichloroethanol-glucuronide (TCE-Glu). Data have been obtained from the cumulative renal excretion in Fig. 5.

by liver alcohol dehydrogenase or oxidized to trichloroacetic acid. It is not known if any biotransformation of chloral hydrate takes place in the gastrointestinal tract. The rate at which trichloroethanol appears in the general circulation is assumed to be a measure for the rate of chloral hydrate absorption.

Marshall and Owens (1954) showed that, only after relatively high oral doses to dogs (> 50 mg/kg), unchanged chloral hydrate could be detected in the plasma for a short period of time. The same was found in horses by Alexander et al. (1967) who gave a total oral dose of 25 g (166 mg/kg). Apparently in these cases saturation of the metabolizing enzyme systems during first passage through the liver had occurred. That the enzymes must have a high capacity in metabolizing chloral hydrate, follows from i.v. experiments in dogs, where a half-life of 3 min was found (Butler, 1948; Garrett and Lambert, 1973). The same result was obtained in our own dog experiment, when testing the applicability of the gas chromatographic assay for chloral hydrate and metabolites (Breimer et al., 1974). In children (4 - 8 yr), Rezza (1956) determined a half-life of about 10 min after i.v. administration of chloral hydrate. The results of Bernstine et al. (1957), who reported chloral hydrate blood concentrations as high as 30 mg/l 12 h after rectal administration of 1.2 g to human subjects, are contradictory with the results of other investigators and are not substantiated by our own studies on chloral hydrate kinetics and metabolism in man.

Trichloroethanol

The initial relatively rapid decline of trichloroethanol blood concentration (Fig. 2) is probably due to a distribution process into fluids and tissues outside of the blood. Its low molecular weight and its relatively good lipophility suggests that extensive distribution could be expected. A further indication for a rapid distribution process lies in the fact that in the two subjects who reached their maximum concentration only after 60 min, no clear biphasic decay was observed. This indicates that now distribution of trichloroethanol occurred more rapidly than chloral hydrate absorption.

The final exponential decay of trichloroethanol blood concentration is due most likely to the metabolic conversion into its glucuronide and also to the oxidation to trichloroacetic acid. The concentration of the latter compound increases up to 12 h. The mean trichloroethanol blood half-life of 8.0 h is consistent with the plasma data of Sellers et al. (1972), who reported 8.2 h. However, in the latter study plasma concentrations were followed only until 6 hours after administration, while in our study this was done until 11 or 12 h. Both periods of time are relatively short with respect to a half-life of 8 h and a

question of the reliability of this half-life after 12 h occurs. In some subjects the trichloroethanol concentrations at 24 h were measured and these appeared to be slightly higher than expected on basis of an 8 h blood half-life. In dogs Garrett and Lambert (1973) also found a slower third phase, which they attributed to the existence of a third or "deep" compartment, from which the rate of return of trichloroethanol to the general circulation is slow. If on the other hand this phase in humans would be very much slower than that corresponding to a half-life of 8 h, then the urinary excretion rate curves or excretion deficit curves of trichloroethanol and possibly also of trichloroethanol-glucuronide should show a distinct second slower phase. This has not been observed in our experiments, although the urine half-lives are slightly longer than the blood half-lives. It can be concluded that if a slower phase in the elimination of trichloroethanol exists, it is probably not much slower than that corresponding to an average half-life of 10 h (* see note p. 284).

Trichloroethanol-glucuronide

The rapid rise of trichloroethanol-glucuronide blood concentration after oral intake of chloral hydrate indicates, that conjugation already occurs to a great extent during first passage of trichloroethanol through the liver. Otherwise, the peak level time of the glucuronide could not coincide with unconjugated trichloroethanol. A further indication for such a "first-pass effect" lies in the fact that the half-life of the glucuronide was found to be shorter than that of free trichloroethanol. According to theory a metabolite cannot be eliminated faster than it is produced from its precursor, unless such a great amount is formed during the first liver passage that it is initially kinetically independent of the precursor. Later, a slower phase would be expected when the rate of formation becomes ultimately the rate-determining step for elimination. There is no direct indication for such a behaviour in our experiments, although the urine half-lives are longer than the blood half-lives. The average urine half-life of the glucuronide is in close agreement with the average blood half-life of unconjugated trichloroethanol, but the agreement for each individual is not so evident.

The kinetic behaviour of trichloroethanol-glucuronide in these experiments is difficult to understand, unless one assumes that during the first passage through the liver by far the greater part of this product is being formed. The initial rapid decline in blood concentration must be due to distribution into a deeper compartment and it is in agreement with similar findings in the dog (Garrett and Lambert, 1973). The reason why such a phase also appears after rectal administration, remains unclear (Fig. 3). In one subject, receiving chloral hydrate

rectally, the glucuronide concentrations were so irregular that no half-life could be determined.

Trichloroacetic acid

Another effect of chloral hydrate administration is the rapid formation of trichloroacetic acid. After oral or rectal intake it seems likely that during first passage of chloral hydrate through the liver a certain part is oxidized by aldehyde dehydrogenase and possibly also by the mixed function oxygenase system. The slower increase in trichloroacetic acid blood concentration, up to 12 h, must be due to the oxidation of trichloroethanol. When the latter compound is administered, there is also a substantial formation of trichloroacetic acid, although its formation rate is slower than after choral hydrate intake (Fig. 4). The half-life of trichloroacetic acid is very long, a period of 100 h in subject A.H. was measured. Paykoç and Powell (1945) found 80 - 100 h after i.v. infusion of the sodium salt to six human volunteers. This large value may be due to the high binding of the compound to human plasma proteins: 94 - 84% in the concentration range 30 - 100 mg/l plasma respectively (Sellers and Koch-Weser, 1971).

During repetitive administration of chloral hydrate, trichloroacetic acid accumulated substantially as can be seen from Fig. 8. Concentrations up to 100 mg/l blood were measured after four daily dosages of 1275 mg. In a hospitalized

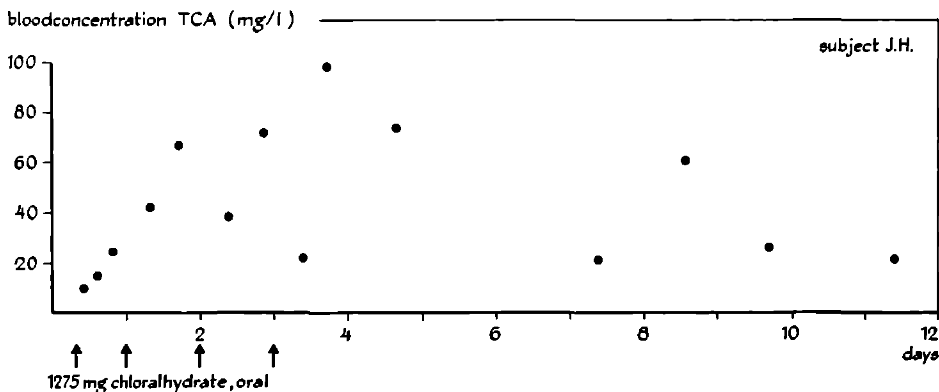

FIG. 8. Blood concentrations of trichloroacetic acid following four oral doses of chloral hydrate (1275 mg), administered with an interval of approximately 24 h. Note the accumulation of trichloroacetic acid in the blood and the strongly fluctuating concentration.

patient, who received chloral hydrate repetitively for more than a fortnight, the trichloroacetic acid blood levels were determined each day at the same time; these varied from 48.0 to 83.3 mg/l. In spite of presumed steady state conditions there was still a great fluctuation in trichloroacetic acid concentration. These findings are in agreement with those of Owens and Marshall (1955), but no satisfactory explanation for this phenomenon is available yet.

Trichloroacetic acid has no hypnotic properties of its own (Marshall and Owens, 1954). The toxicity of this compound is not known. However, it is one of the strongest organic acids known and its use in vitro as a protein precipitant suggests that it may be harmful at certain concentrations. This becomes the more important when such a compound accumulates in relatively high concentrations in the body during chronic administration of the parent drug. It is possible that protein binding protects the organism against toxic effects. The substantial binding to plasma proteins on the other hand may cause displacement of other drugs from their binding sites at human albumin as has been shown for warfarin (Sellers and Koch-Weser, 1970; ib., 1971). Cucinell et al. (1966) attributed the decreased half-life of coumarins in subjects receiving chloral hydrate to an induction of drug metabolism. In this instance, however, displacement from albumin may also be involved. The net effect of such interaction cannot always be predicted, as a greater amount of free drug initially causes potentiation of activity, which is on the other hand counteracted by an increased rate of elimination.

Although no literature data are available about unwanted effects of trichloroacetic acid, apart from interaction with other drugs at the protein binding sites, it must be emphasized that the formation of such a metabolite is highly undesirable.

The overall fate of chloral hydrate in the body

In pharmacokinetic and metabolic studies of chloral hydrate previous attention has been given to three metabolites; trichloroethanol, trichloroethanol-glucuronide and trichloroacetic acid. After perusal of the total urinary excretion of these metabolites, it is apparent that only part of the administered dose can be accounted for (Table IV). Although only part of trichloroacetic acid excretion was measured, it seems unlikely that the rest was responsible for the remaining amount of the dose to be recovered. Owens and Marshall (1955) found that in some subjects excretion of trichloroethanol-glucuronide and of trichloroacetic acid could account for nearly the entire dose of chloral hydrate, while in other subjects this was only a small fraction. They also found that after continued daily administration of chloral hydrate the percentage

excreted as trichloroacetic acid in 24 h varied substantially, not only in different individuals but also in the same individual. This latter fact seems consistent with the fluctuating blood levels of trichloroacetic acid which was mentioned earlier. From the data of Paykoç and Powell (1954) it can be concluded that at least 90% of the administered dose of trichloroacetate anion was excreted unchanged in the urine. Garrett and Lambert (1973) showed that in the dog only trace amounts of the metabolites were excreted via faeces. Initially, about 5% of the glucuronide was secreted in the bile, but hydrolysis occurred and trichloroethanol was reabsorbed. No data on faecal excretion in humans are available. It seems likely that unknown pathways are involved in the elimination of chloral hydrate and of trichloroethanol. This could occur by other metabolic routes and also by excretion via the lungs or in the sweat. Further investigations are required in order to elucidate the overall fate of chloral hydrate in the body.

CONCLUSIONS

After oral and rectal administration of chloral hydrate the hypnotic activity must entirely be attributed to the active metabolite trichloroethanol. From the pharmacokinetic point of view chloral hydrate seems to be a suitable drug for hypnotic drug therapy, as the half-life of trichloroethanol is no longer than 10 h. It is difficult to state whether an oral dose of 15 mg/kg is sufficient to induce and maintain sleep. In these studies no definite effects of CNS-depression became apparent. Combining our data with clinical studies it might be concluded that the minimum effective trichloroethanol blood concentration for adults is in the range of 4 - 7 mg/l.

The formation of trichloroacetic acid, after chloral hydrate administration, is a matter of concern. More information is required about the possible harmful actions of this metabolite, especially during chronic use of chloral hydrate. Trichloroacetic acid is highly bound to plasma proteins and therefore, chloral hydrate should not be combined with drugs that also show substantial protein binding.

Chloral hydrate is usually administered orally, but it causes irritation to mucous membranes. Therefore, it is generally recommended that the drug is greatly diluted wtih water or milk in order to avoid gastric irritation (Martindale, 1972). Chemical derivates have been prepared which are claimed not to cause the unpleasant side-effects and which can be dispensed in tablet form. They release chloral hydrate or trichloroethanol in the stomach or small intestine, e.g. chloral betaine, dichloralphenazone and triclophos (Sharpless, 1970; Sellers et al., 1973).

Quite often, chloral hydrate is administered rectally in enemas or suppositories. This is of special advantage with respect to children, as it avoids the irritation and bad taste of oral preparations.

Few biopharmaceutical studies haven been performed concerning the ability of the different dosage forms in delivering the drug to the bloodstream. Such data are important for assessing the effectiveness of a certain preparation in the treatment of insomnia and convulsions. The rate of absorption and the bioavailability, both related to trichloroethanol blood concentrations, are important in this respect. In fact detailed information is available only for an aqueous solution after oral intake, from which the drug is rapidly absorbed (Marshall and Owens, 1954; Sellers et al., 1972). In our study on the pharmacokinetics of chloral hydrate it was found that peak concentrations of trichloroethanol were reached 20 - 60 min after administration of the parent drug in aqueous solution (Table I, p. 255).

In view of the fact that no in vivo data were available on the rectal absorption of chloral hydrate, it was decided, firstly to study some rectal dosage forms. In addition, it was considered pertinent to study two oral capsule preparations, which are supplied in the Netherlands under the trade names of CHLORALDURAT-Red and CHLORALDURAT-Blue.

1. RECTAL ADMINISTRATION

INTRODUCTION

Despite the widespread use of rectal application of chloral hydrate, no data on the effectiveness of rectal absorption have appeared until now. The purpose of this investigation was to study the absorption of chloral hydrate by the rectal

route and to establish the influence of vehicle composition. It has been discussed already (refer to "pharmacokinetics and metabolism" of this chapter) that the initial rise of trichloroethanol concentration in blood is related to the absorption rate of chloral hydrate. Also, bioavailability calculations will be based on trichloroethanol blood concentrations.

A review of the various dosage forms for rectal administration has been published by Cox (1971). For the present study four different pharmaceutical formulations, two types of suppositories and two types of enemas, were chosen:

1. suppository with 1000 mg chloral hydrate dissolved in 2.20 g Estarine D;
2. suppository with 1000 mg chloral hydrate dissolved in a mixture of 2.08 g polyethylene glycol 1540 and 0.52 g polyethylene glycol 6000;
3. rectiole with 1100 mg chloral hydrate dissolved in 2.16 g sesame oil;
4. rectiole with 1100 mg chloral hydrate dissolved in 2.67 g polyethylene glycol 300.

Details on the preparation of these dosage forms, as well as an outline of the reasons why these preparations were chosen, have been described elsewhere (Breimer, Cox and van Rossum, 1973).It should be mentioned here that the rectioles contained 100 mg of excess chloral hydrate, because it was estimated that this amount was left behind after administration.

METHODS

The dosage forms were prepared at the Dutch Pharmacist's Laboratory, The Hague, and they were used for the in vivo studies 2 - 6 weeks after preparation. The chloral hydrate content was determined according to a modified method described by Schoorl (1929). The sesame oil rectioles and the PEG-rectioles contained 100% and 101% respectively; the Estarine D suppositories and the PEG-suppositories contained 96% and 102% respectively.

The subjects who participated in the study were healthy male volunteers, ranging in age from 20 - 25 years and in weight from 67 - 83 kg. They had not received regular medication during the 4 weeks preceding initiation of the experiments. The subjects had fasted overnight prior to the trial and for 3½ hours after administration of the drug preparation. At 9 a.m. the chloral hydrate dosage form was introduced into the rectum and blood samples were taken from a forearm vein, usually at ¼, ½, 1, 1½, 2, 3, 5, 7, 9 and 12 hours after administration. Sometimes a sample had to be omitted due to organization problems. The blood was drawn into tubes containing a small drop of heparin

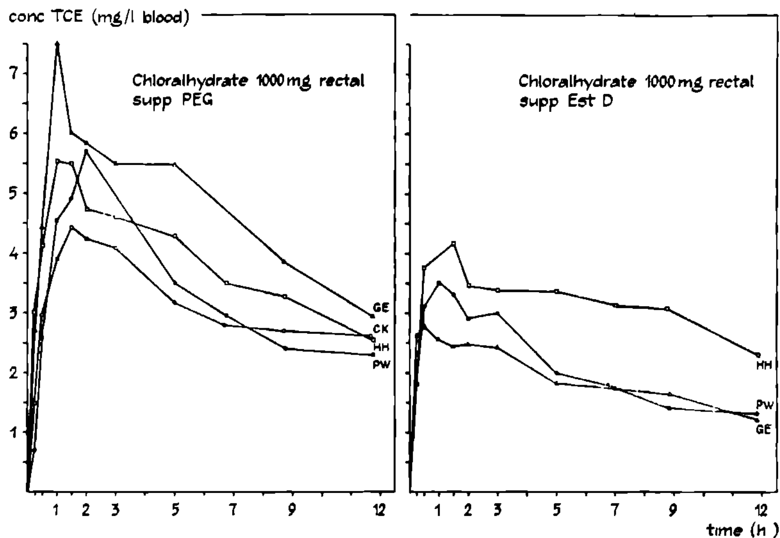

FIG. 9. Individual trichloroethanol blood concentration curves following rectal administration of 1000 mg chloral hydrate in two types of suppositories.

solution as anticoagulant. Immediately after taking the blood 1.0 ml was introduced into a 25.0 ml glass vial, containing 1 ml of lead acetate solution and the free trichloroethanol blood concentration was determined as described in Chapter 9, Section II.

Early removal of the rectum contents by defaecation was never experienced. For those subjects who participated twice in the investigation, there was at least an interim of 7 days between the two consecutive experiments.

RESULTS

The individual curves obtained are shown in Fig. 9 (suppositories) and Fig. 10 (rectioles). Unfortunately, two subjects in the study with the rectioles and one with the suppositories were not able to participate more than once in the study. In Fig. 11 the curves per individual, who received two different dosage forms, are given. There is a significant variation in absorption rates and blood levels between the individuals per dosage form. Between lipophilic bases and PEG bases there are great differences in concentration values. A rapid initial rise in trichloroethanol occurred in all subjects receiving a suppository. Absorption was fast and peak concentrations were reached between ½ and 2 hours after ad-

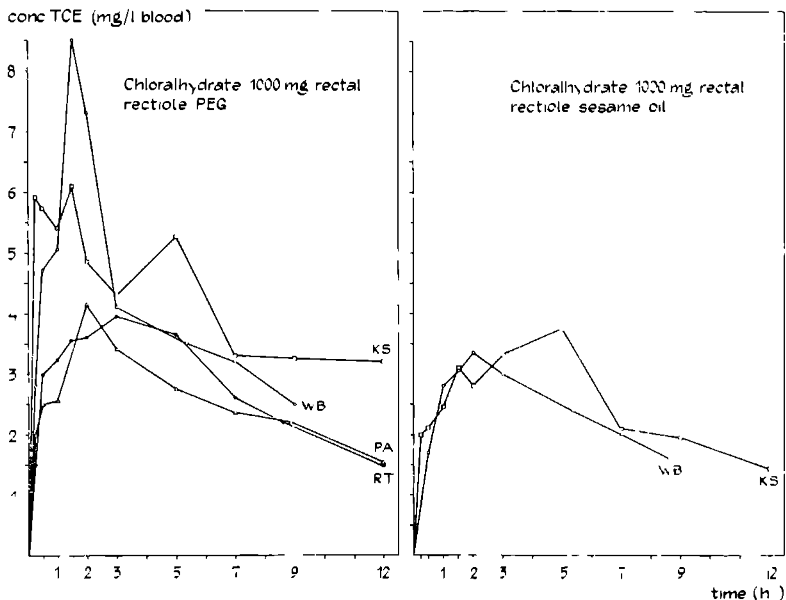

FIG. 10. Individual trichloroethanol blood concentration curves following rectal administration of 1000 mg chloral hydrate in two types of rectioles.

ministration. If absorption is regular and takes place according to a first-order process, as is often the case, the half-life of absorption ($t_{1/2a}$) is characteristic for the absorption rate or rate of availability. The dose, the $t_{1/2a}$ and $t_{1/2el}$ determine the t_{max} and C_{max}, which can be calculated.

However, a careful analysis of the concentrations in the first few hours reveals that in most cases the absorption process cannot be described accurately by a first-order kinetic process, and therefore it seems ill-advised to calculate these parameters. Consequently one can obtain only an overall impression of the time necessary to reach the maximum concentration. It seems that both suppository bases are able to deliver the drug with approximately the same rate to the blood stream. With the PEG-rectioles absorption also appears to be rapid in two subjects, while in the other cases absorption is slower and takes place over a longer period of time. The slow absorption from the sesame oil rectiole is striking.

Bioavailability refers to the fraction of the dose of a drug administered that reaches the general circulation intact. In the case of chloral hydrate the amount becoming available is related to trichloroethanol, as this is the compound measured in vivo. The following may be derived:

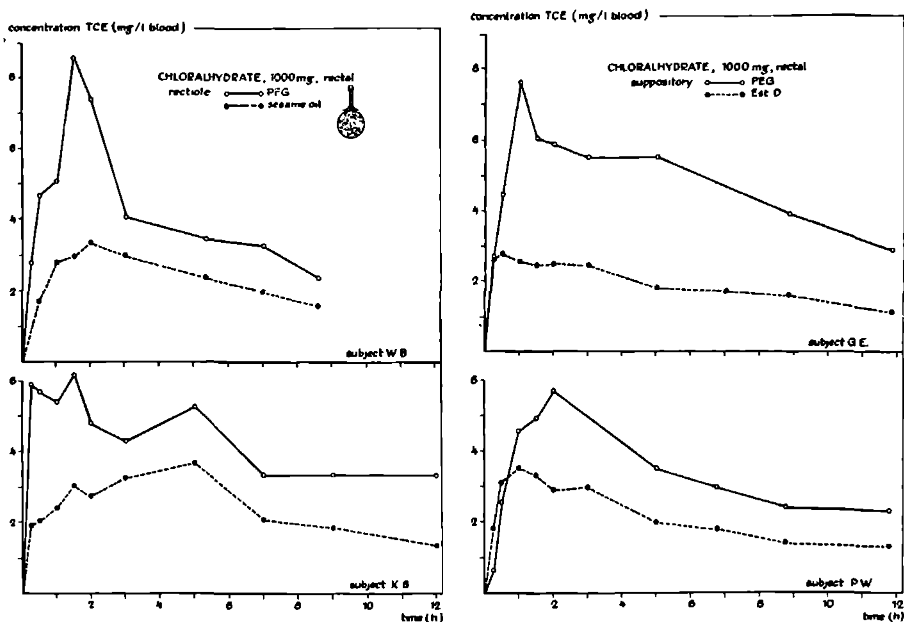

FIG. 11. Comparison of individual trichloroethanol blood concentration curves following rectal administration of 1000 mg chloral hydrate in different vehicles to the same subjects.

$$F \times D = k_{el} \cdot \int_{0}^{\infty} C_p \, dt = k_{el} \cdot AUC$$

in which:

F = bioavailability ($0 \leqslant F \leqslant 1$),
D = dose administered,
C_p = blood or plasma concentration,
k_{el} = total body clearance constant,
AUC = area under the plasma concentration-time curve from $t = o$ to $t = \infty$.

The k_{el} of trichloroethanol can be determined only after intravenous administration and therefore the absolute bioavailability cannot be determined if only rectal data are available. Moreover, the dose in this case is related to chloral hydrate and not to trichloroethanol, so that the fraction of the dose that is converted to trichloroethanol also remains uncertain.

However, it is a reasonable assumption that in the same individual this fraction is constant and also that k_{el} for trichloroethanol remains constant.

TABLE VI. Relative bioavailability of chloral hydrate from rectioles and suppositories in the same individual after rectal administration.

Subject	Body-weight (kg)	Dosage form	Dose* (mg)	Dose/kg (mg)	AUC 0-12 h (mg.h/l)	conc. 12 h (mg/l)	Total AUC 0 - ∞ (mg.h/l)	F_{rel} (%)
P.W.	75	supp. PEG	1000	13.3	36.5	2.3	69.8	100
		supp. Est D	1000	13.3	22.2	1.3	40.9	59
G.E.	82	supp. PEG	1000	12.2	51.8	2.9	93.6	100
		supp. Est. D	1000	12.2	20.1	1.2	37.1	40
H.H.	75	supp. PEG	1000	13.3	41.0	2.6	78.4	100
		supp. Est. D	1000	13.3	32.5	2.3	65.7	84
K.S.	67	rect. PEG	1000	14.9	45.4	3.4	94.4	100
		rect. SO	1000	14.9	27.2	1.4	47.8	51
W.B.	83	rect. PEG	1000	12.0	33.4	2.4 (9 h)	68.0	100
		rect. SO	1000	12.0	19.6	1.6 (9 h)	42.8	63

*) These values have not been corrected for the assayed chloral hydrate content of the dosage forms, since the deviations did not exceed ± 5%.

Then the relative bioavailability of chloral hydrate from a certain dosage form (I) can be determined by comparison to a reference dosage form (II) in the same subject. The following now applies:

$$F_{rel} = \frac{AUC_I}{AUC_{II}}$$

If the AUC is not determined to t = ∞, then it must be corrected to include the undetermined AUC. This is quite possible assuming that at the last data point, at time t = t′, absorption has been completed, using Dost's law of corresponding areas (Dost, 1968). The infinite area is given by:

$$\int_0^\infty C_p\,dt = \int_0^{t'} C_p\,dt + \int_{t'}^\infty C_p\,dt = AUC\Big/_0^{t'} + C_p^{t'} \cdot \tau_{el}$$

in which τ_{el} is the overall elimination time constant and $C_p^{t'}$, the last blood concentration measured for the determination of AUC from t = 0 to t = t′.

The AUC's in the experiments of this investigation were determined by

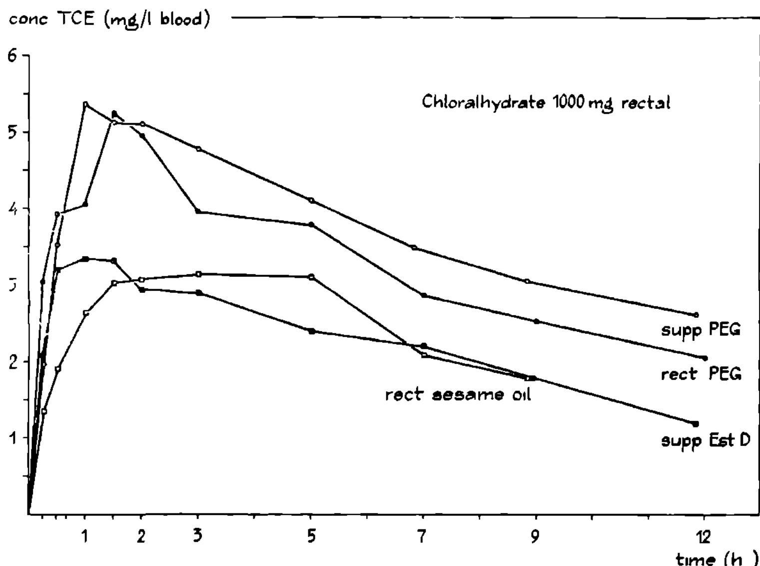

FIG. 12. Average trichloroethanol blood concentration curves after rectal administration of the four dosage forms, each containing 1000 mg of chloral hydrate.

weighing the corresponding areas under the blood concentration-time curves up to 12 hours after administration. The remaining area was calculated by multiplying the concentrations at 12 hours by the overall elimination time constant τ_{el}. Because of irregularities in absorption it was not possible to determine an exact τ_{el} ($\tau_{el} = 1.44 \times t_{1/2}$) in each subject and it was decided to take one value for all individuals: $t_{1/2} = 10$ hours $\rightarrow$ $\tau_{el} = 14.4$ hours. Results per individual will hardly be influenced by this assumption.

In Table VI the results are given for the five subjects to whom two dosage forms were administered. In all cases the PEG vehicles resulted in a higher bioavailability and they were considered as the reference dosage form. These data show clearly a lower bioavailability per individual of the suppositories with Estarine D and the rectioles with sesame oil.

In Fig. 12 the mean plasma concentration curves for all four dosage forms are given and their bioavailability is calculated relative to the PEG suppository (Table VII). Although the number of subjects is limited, it can be seen that the suppositories and rectioles with PEG are both quite efficient, whereas the Estarine D and sesame oil are both unsatisfactory. These data confirm the results in the individual subjects. So the oily vehicle for the rectal administration

TABLE VII. Relative bioavailability of chloral hydrate from rectioles and suppositories after rectal administration (mean values).

No. subjects	Dosage form	Dose (mg)	Dose/kg (mg)	AUC 0-12 h (mg.h/l)	conc. 12 h (mg/l)	Total AUC 0 - ∞ (mg.h/l)	F_{rel} (%)
4	supp. PEG	1000	13.7	44.4	2.6	82.1	100
3	supp. Est. D	1000	12.9	27.1	1.6	50.1	61
4	rect. PEG	1000	14.3	38.6	2.3	69.3	84
2	rect. SO	1000	13.5	23.7	1.8 (9 h)	49.3	60

of chloral hydrate seems to be an inferior delivery system compared to the hydrophilic PEG system.

DISCUSSION

The design and purpose of the study were, for practical reasons, very simple: comparison of the same dosage form, but with two different vehicles, in the same subject after rectal administration. This allows relative bioavailability measurement per individual under the assumptions mentioned. Unfortunately some of the volunteers were not able to participate in the study for a second time, so that only part of the design of the experiments could be realized. Nevertheless the results are sufficiently important to be reported, although the conclusions drawn must be considered against the background of a limited number of observations.

Some individual curves are given in Fig. 11. Representation of the results in this way immediately gives an impression of the differences between dosage forms. It is also important to show all the individual curves as this gives insight into the variability between different individuals. The differences are quite great as can be seen from Fig. 9 and Fig. 10. Often in the literature only mean data are reported and these are then treated mathematically. The results of such a manipulation are given in Fig. 12 and it may be noted that smoother curves are obtained in this way. Irregularities in absorption are not reflected in these mean curves, whereas they are obvious from the individual ones. Information is lost therefore by giving only mean values.

The results indicate a poor bioavailability of chloral hydrate from the lipophilic vehicles sesame oil and Estarine D, compared to the hydrophilic PEG.

The reason for these differences is not clear. In the rectioles, and probably also in the suppositories, chloral hydrate is dissolved in the vehicle. Consequently, the differences cannot be due to particle size. Chemical incompatibilities with chloral hydrate are reported for many substances including sesame oil, Carbowax (a PEG brand) (Fairbrother, 1973) and the suppository bases Witepsol and Imhausen (Petrićić and Jalsenjak, 1971) which are chemically related to Estarine D. It seems likely that "chemical incompatibility" influences the extent of chloral hydrate release from the different bases to varying degrees, probably depending on the nature of interaction between chloral hydrate and the base. The in vitro disintegration results (Breimer, Cox and van Rossum, 1973) of the suppositories do not give any further explanation. On the contrary, as far as disintegration is concerned the in vitro results do suggest a better absorption from the lipophilic base.

Another important factor is the rate at which the active compound becomes available in the general circulation. This seems to be relatively high for PEG suppositories and much slower for sesame oil rectioles. The absorption rate of chloral hydrate from PEG suppositories is lower than after oral administration of an aqueous solution, but higher than from CHLORALDURAT-Red capsules (Fig. 13, p. 279).

Central nervous system (CNS) depressant effects after administration of chloral hydrate in these dosage forms are difficult to assess objectively as the subjects received the drug in the morning, just after waking up. Generally no definite feelings of CNS depression became apparent. However, two subjects (W.B., Fig. 10 and G.E., Fig. 9) did report feelings of drowsiness at the time of trichloroethanol peak concentration (8.5 and 7.5 mg/l respectively).

There were no complaints about irritation of the rectum after administration of these dosage forms and as already mentioned in no instance was the defaecation reflex initiated. For one subject, who was suffering from haemorrhoids, the Estarine D suppository was irritating while the PEG suppository was not.

Of the dosage forms used in this study, the rectioles and the PEG suppositories are the easiest to prepare. A disadvantage of the rectioles is that the quantity administered is probably less exact. From the stability point of view, sesame oil rectioles and PEG suppositories are preferable (Cox, 1973).

CONCLUSIONS

The PEG suppository must be regarded as the best rectal chloral hydrate delivery system of the dosage forms investigated. Its high bioavailability and

rapid absorption make it suitable for the treatment of insomnia and possibly also in the treatment of convulsions. When using this dosage form in children, the dose has to be reduced according to the age of the child.

2. ORAL ADMINISTRATION

INTRODUCTION

Oral administration of chloral hydrate in an aqueous solution or in an elixir must be considered as an effective method of treating insomnia. Peak concentrations of trichloroethanol are generally reached within one hour, which makes it highly probable that at an earlier time the minimum effective concentration is exceeded, provided that an appropriate dose has been given.

As already mentioned, in the Netherlands chloral hydrate is also supplied in gelatine capsules of 250 mg under the trade name CHLORALDURAT. There are two types available: CHLORALDURAT-Red, which is claimed to be suitable for the induction of sleep (dose recommended 2 - 4 capsules) and CHLORALDURAT-Blue which is claimed to be suitable in the treatment of early morning awakening (dose recommended 2 - 3 capsules). From the latter capsules the release of chloral hydrate is supposed to take place about 4 hours after intake (Pohl-Boskamp, information sheet on Chloraldurat). However, no blood levels have been measured to substantiate these claims and even in vitro data are lacking in the literature. Because of the general lack of information about these dosage forms, it was decided to perform a biopharmaceutical study on these preparations, in vivo and in vitro. The in vitro dissolution studies have been carried out in the Dutch Pharmacist's Laboratory, The Hague and details will be reported elsewhere.

METHODS

The CHLORALDURAT dosage forms were commercially obtained. The chloral hydrate content was determined at the laboratory of the hospital pharmacy, St. Radboudziekenhuis, Nijmegen, according to a modified method by Schoorl (1929). CHLORALDURAT-Red contained 250 mg (100%) of chloral hydrate and CHLORALDURAT-Blue 240 mg (96%). Healthy male volunteers, ranging in age from 20 - 25 years and in weight from 55 - 80 kg, participated in the study. They had not received regular medication during the 4 weeks preceding initiation of the study. The subjects had fasted overnight and those

receiving the CHLORALDURAT-Red capsules were not allowed to take food or fluid for 3.5 h after administration. A 5.5 h period was applied similarly for CHLORALDURAT-Blue. At 9 a.m. 4 capsules of chloral hydrate, 250 mg each, were administered orally together with 150 ml water. The subjects were asked to remain in an upright position for about 15 min and then to lie down for 3.5 h (Red-capsules) or 5.5 h (Blue-capsules) periods. This procedure was undertaken in order to simulate as closely as possible the situation of taking a hypnotic drug and going to bed. Blood samples were taken from a forearm vein, usually at the following times when CHLORALDURAT-Red was given: ¼, ½, 1, 1½, 2, 2½, 3, 4, 5, 7, 9 and sometimes at 12 h; when CHLORALDURAT-Blue was given at times of 1, 2, 3, 4, 4½, 5, 6, 7, 9 and 12 h approximately. The blood was drawn in tubes containing a small drop of heparin solution as anticoagulant. Immediately after collection of the blood 1.0 ml was introduced into a 25.0 ml glass vial, containing 1 ml of lead acetate solution, and the free trichloroethanol blood concentration was determined as described in Chapter 9, Section II.

RESULTS

The individual blood level curves obtained are shown in Fig. 13 (CHORALDURAT-Red) and in Fig. 14 (CHORALDURAT-Blue). A careful analysis of the concentrations in the first few hours reveals that in most cases the absorption behaviour cannot be described accurately by a first-order kinetic process. An overall impression about the absorption rate can be obtained by looking at the time required to reach the maximum blood concentration (Table VIII and IX). In three individuals relatively high blood concentrations were achieved after intake of the Red-capsules, but the peak concentrations were reached only after 2 - 4 h. In two subjects receiving this dosage form absorption, apparently, occurred slower or to a lesser extent. With the Blue-capsules the trichloroethanol blood concentration slowly increased up to 3 - 5 hours. After that, in three subjects the blood concentration quite suddenly increased. The peak concentrations ultimately reached were lower than with the Red-capsules and the peak times varied widely. In two subjects no such a change in the blood concentration profile was observed and the trichloroethanol concentrations remained very low at all times up to 12 h.

In Fig. 15 the mean curves for the two preparations are given and these permit a general view on their blood level profile. However, it should be realized that they are composed of strongly varying individual curves, which should be considered as more valid for the practical situation.

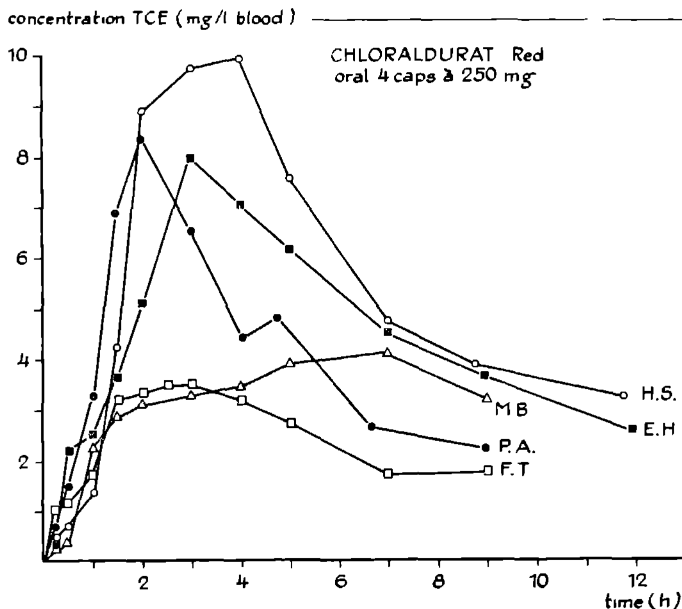

FIG. 13. Individual trichloroethanol blood concentration curves following oral administration of four CHLORALDURAT-Red capsules (1000 mg chloral hydrate).

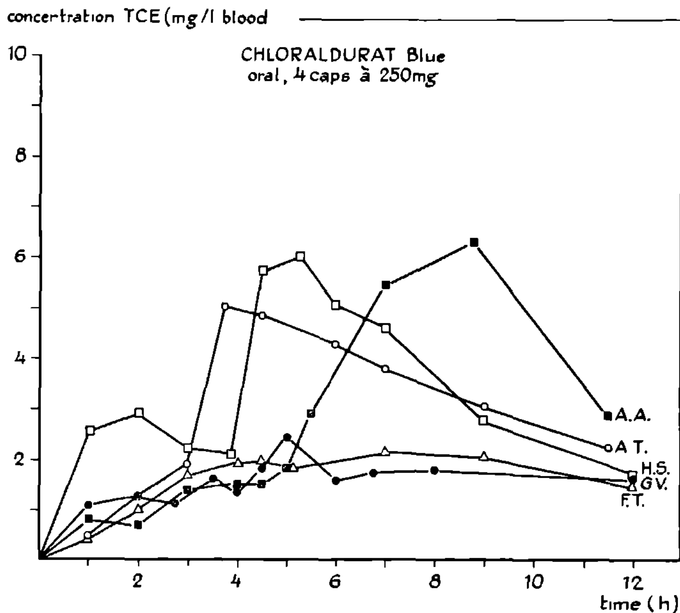

FIG. 14. Individual trichloroethanol blood concentration curves following oral administration of four CHLORALDURAT-Blue capsules (1000 mg chloral hydrate).

TABLE VIII. CHLORALDURAT-Red absorption characteristics after oral administration.

Subject	Body-weight (kg)	Dose (mg)	Dose/kg (mg)	t_{max} [1] (h)	C_{max} [2] (mg/l)	AUC 0 - 9 h (mg.h/l)	conc. 9 h (mg/l)
P.A.	64	1000	15.6	2.0	8.4	40.1	2.2
H.S.	58	1000	17.2	4.0	9.9	63.2	3.8
M.B.	59	1000	17.1	7.0	4.1	30.1	3.2
E.H.	55	1000	18.2	3.0	8.0	51.2	3.6
F.T.	80	1000	12.5	2.5	3.5	23.4	1.8
mean	63	1000	16.1	3.7	6.8	41.6	2.9

mean AUC 0 - ∞ : $41.6 + (2.9 \times 14.4) = 83.4$ mg.h/l

[1]) Time after which the maximum trichloroethanol blood concentration was reached.
[2]) The maximum trichloroethanol blood concentration.

TABLE IX. CHLORALDURAT-Blue absorption characteristics after oral administration.

Subject	Body-weight (kg)	Dose (mg)	Dose/kg (mg)	t_{max} (h)	C_{max} (mg/l)	AUC 0 - 12 h (mg.h/l)	conc. 12 h (mg/l)
G.V.	71	1000	14.1	no real max.		16.8	1.6
A.T.	65	1000	15.4	3.75	5.0	33.4	2.2
F.T.	78	1000	12.8	no real max.		19.2	1.4
A.A.	80	1000	12.5	8.75	6.3	34.3	2.9
H.S.	58	1000	17.2	5.25	6.0	40.5	1.8
mean	70	1000	14.4	5.55	5.5	28.9	2.0

mean AUC 0 - ∞ : $28.9 + (2.0 \times 14.4) = 57.7$ mg.h/l

relative bioavailability Blue/Red: $\frac{57.7}{83.4} \times 100\% = 69\%$

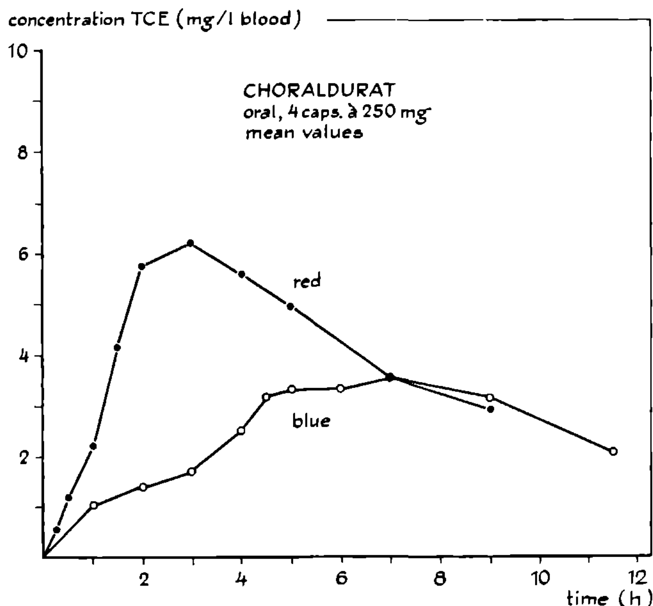

FIG. 15. Average trichloroethanol blood concentration curves after oral administration of CHLORALDURAT-Red and CHLORALDURAT-Blue to five subjects.

The AUC's from t = 0 to t = ∞ in these experiments were determined in the same way as described for rectal administration. If it is assumed that in the two groups of volunteers the average amount of chloral hydrate converted to trichloroethanol has been the same and also that the mean clearance for trichloroethanol was the same, then the mean AUC's will give reliable information about the relative bioavailability of chloral hydrate from the two preparations. It was calculated that this is 69% for CHLORALDURAT-Blue, relative to CHLORALDURAT-Red (Table IX). In the two individuals who received both dosage forms, these values were 56% (H.S.) and 82% (F.T.).

DISCUSSION

The purpose of this study was to obtain information about the rate of absorption of chloral hydrate from the two CHLORALDURAT dosage forms. This parameter is important in relation to the use of these preparations; sleep induction (Red) and sleep continuation (Blue). No special attempt therefore was made to study both preparations in the same individuals. It was hoped

that sufficient information would be obtained from different subjects. The mean AUC values would provide an indication of relative bioavailability.

It is evident that rapid absorption of a hypnotic drug from its dosage form is required if it is intended to be used in patients who drop off too late. It could be claimed, in this respect, that the time necessary to reach a minimal effective blood concentration, should not exceed 30 min. After studying the initial rise in trichloroethanol blood concentration, when CHLORALDURAT-Red is given, it can be observed that an effective concentration is not reached in any of the subjects, even within a period of one hour. Despite the relatively high concentrations which are reached ultimately in the three subjects receiving this dosage form, it is concluded that CHLORALDURAT-Red is not a suitable preparation for the rapid induction of sleep. Consequently, if chloral hydrate is required for this purpose, an aqueous solution of this drug should be used. The reason why, in two of the subjects, the blood levels were much lower than in the other three, remains unclear. It is possible that release of the drug from the capsules was not complete in these subjects. It is possible also, that fasting regulations were not obeyd, so that food is another interfering factor.

With respect to CHLORALDURAT-Blue it is clear that some chloral hydrate is released at an early time. The release sharply increases after 3 - 5 h in some subjects, since the concentration of trichloroethanol in blood rapidly rises during this time. The concentrations ultimately reached are lower than with CHLORALDURAT-Red and it remains uncertain whether these are sufficient to maintain sleep. This is even more doubtful when the recommended doses of 250 - 750 mg are given. There is also a possibility, with these capsules, that the peak concentration time is attained long after a period of 4 h. In subject A.A. this occurred 9 h after administration, which is a period when the subject had to begin daily work. Drowsiness and hang-over effects are to be expected. In two other subjects no sharp increase in trichloroethanol blood concentration occurred. The reason for this could again be an incomplete disintegration or release from the capsules or the influence of food. It is striking that in subject F.T., who received both dosage forms, absorption was incomplete on both occasions (Fig. 16). After enquiries, when the results were available, this subject denied having consumed food before the experiments. It might be that some physiological cause is underlying this observation. In conclusion, CHLORALDURAT-Blue is not a very reliable dosage form with respect to the treatment of early morning awakening. However, CHLORALDURAT-Red, although not meant for this purpose, could be used instead. Results show that blood concentrations between 4 and 6 h after administration of Red-capsules are at least as high as the Blue peak concentrations. Besides, there is less risk of residual effects with Red-capsules the following morning.

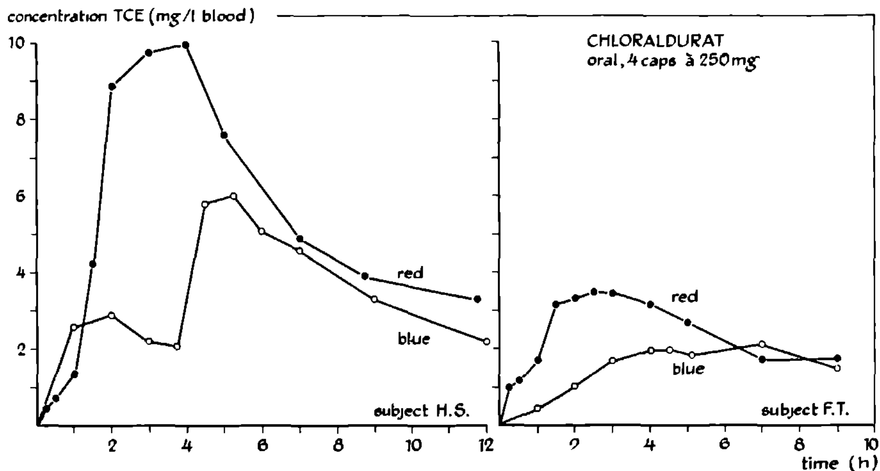

FIG. 16. Comparison of individual trichloroethanol blood concentration curves following oral administration of CHLORALDURAT-Red and CHLORALDURAT-Blue to the same subjects.

The lower bioavailability of CHLORALDURAT-Blue indicates that a smaller fraction of chloral hydrate is released from these capsules in the gastrointestinal tract. The in vitro results revealed that the Blue-capsules did not disintegrate completely in artificial gastric or intestinal juice. This in contrast to CHLORALDURAT-Red which disintegrated quite rapidly at pH 7.5.

A further interesting observation has been that the mean AUC of the chloral hydrate aqueous solution is lower than of the Red-capsules: 63.2 mg.h/l (corrected for an average dose of 1000 mg). This implies a relative bioavailability of 76% when compared with the Red-capsules. Further experiments are needed to clarify this observation.

Finally it should be mentioned that none of the subjects complained about gastric irritation after intake of the capsules. Central nervous system depression was only experienced by subject H.S., who also showed the highest trichloroethanol blood concentrations.

CONCLUSIONS

CHLORALDURAT-Red is not a suitable preparation for the rapid induction of sleep, as absorption is too slow. It might however be used in the treatment of early morning awakening, since trichloroethanol blood concentrations are relatively high 4 h after drug intake.

CHLORALDURAT-Blue cannot be regarded as a reliable preparation for the maintenance of sleep, as the blood concentration profile is liable to considerable variation.

For a rapid induction of sleep with chloral hydrate the most reliable preparation is an aqueous solution when orally administered. The second choice is the chloral hydrate PEG-suppository for rectal administration.

SUMMARY

Pharmacokinetics and metabolism

The pharmacokinetics of chloral hydrate and its metabolites were studied in man after oral and rectal administration. Healthy volunteers received oral doses of chloral hydrate (15 mg/kg), dissolved in 150 ml water, or 1000 mg rectally in a rectiole. The subjects, participating in the oral experiments collected their urine for a period of about 50 h. Blood and urine concentrations of trichloroethanol, trichloroethanol-glucuronide and trichloroacetic acid were determined at regular intervals.

Unchanged chloral hydrate did not reach the blood circulation in measurable amounts. The CNS depressant effects, after oral or rectal chloral hydrate administration, are entirely attributed to trichloroethanol. Peak levels of trichloroethanol as well as trichloroethanol-glucuronide were reached 20 - 60 min after oral drug administration. The average blood half-lives of trichloroethanol and the glucuronide were 8.0 h and 6.7 h respectively. The half-lives of these compounds when deduced from the renal excretion deficit curves were slightly longer. After rectal administration absorption was slower, but the half-lives of the two metabolites were in the same range as after oral administration. A third important metabolite of chloral hydrate is trichloroacetic acid. An additional experiment revealed that trichloroethanol administration resulted also in a substantial formation of trichloroacetic acid. The half-life of this metabolite was about 4 days in man and during repetitive chloral hydrate administration considerable accumulation (up to 100 mg/l blood) was observed. Due to its high protein binding, interaction with other drugs should be considered.

Unchanged chloral hydrate could not be detected in the urine and only small amounts of free trichloroethanol were excreted (2.6 - 7.7 mg). The glucuronide excretion amounted to 11 - 24% of the administered dose of chloral hydrate, whereas

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NOTE:

*) Recently a paper by Ertle et al. (1972) on the metabolism of trichloroethylene in man, came to our attention. Trichloroethanol is an important metabolite of this industrial solvent and for comparative reasons these authors included an oral experiment with chloral hydrate (15 mg/kg) administered to two human volunteers. They measured trichloroethanol blood concentrations until 51 h after intake and found a half-life for the slowest phase of about 12 h.

9 - 57 mg trichloroacetic acid were excreted within two days. There may be additional pathways for the elimination of chloral hydrate and trichloroethanol.

It has been concluded that chloral hydrate may be a suitable drug for the treatment of insomnia, although the formation of trichloroacetic acid remains a matter of concern. The properties of this metabolite should be subject of further research.

Biopharmaceutics

1. Rectal administration

The relative bioavailability of chloral hydrate was investigated in man after rectal administration of different dosage forms. The following preparations were used: a rectiole with 1100 mg chloral hydrate dissolved in sesame oil, a rectiole with 1100 mg chloral hydrate dissolved in PEG 300, a suppository with 1000 mg chloral hydrate in a base of PEG 1540/6000 (4 : 1). These dosage forms were administered to healthy volunteers and blood concentrations of trichloroethanol were determined at regular intervals. Relative bioavailability was calculated by measuring the area under the blood concentration-time curve from $t = 0$ to $t = \infty$ (AUC) and comparing it to the AUC obtained with the PEG suppository (100%). The mean value found for the suppository with Estarine D was 61%, for the PEG rectiole 84% and for the sesame oil rectiole 60%. Consequently, the lipophilic bases were found to be less effective chloral hydrate delivery systems when compared with the PEG bases. Absorption from the suppository bases was quite rapid and peak levels were attained after ½ - 2 hours.

It is concluded that in general practice the PEG suppository is the preferable dosage form for rectal administration of chloral hydrate.

2. Oral administration

The preparations of CHLORALDURAT-Red and -Blue were investigated. Four capsules (1000 mg chloral hydrate) were administered to healthy volunteers and blood concentrations of trichloroethanol were determined at regular intervals. Peak concentrations after administration of the Red-capsules were reached after 2 - 4 h and it has been concluded that the absorption from these capsules was too slow, in order to be effective in the treatment of difficulties getting to sleep. CHLORALDURAT-Blue, which is supposed to deliver its active ingredient about 4 h after administration, showed a varying blood level profile with peak concentrations occurring between 3 and 9 h. Bioavailability of the Blue-capsules was estimated to be 69% relative to the Red-capsules. It has been concluded that CHLORALDURAT-Blue is not a reliable preparation to be used in hypnotic drug therapy. CHLORALDURAT-Red may be used for sleep maintenance.

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SECTION IV

PHARMACOKINETICS AND LIVER DISEASE*

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PHARMACOKINETICS OF HEXOBARBITAL IN PATIENTS WITH LIVER DISEASE

INTRODUCTION

In the biotransformation of drugs the liver plays the major role. Until recently it was considered that drug metabolism was impaired in patients with liver disease, which resulted in an increased sensitivity of these patients towards drugs which are primarily inactivated by metabolism (Sherlock, 1963; Bush, 1963). However, the evidence for this supposition is rather conflicting (Brodie et al., 1959; Sherlock, 1968). Patients with chronic liver disease have failed to exhibit delayed biodegradation of such drugs as dicumarol, salicylates and antipyrine (Brodie et al., 1959). Only some of the patients with impaired liver function displayed a slower disappearance for phenobarbital and phenytoine (Kutt et al., 1964), phenylbutazone (Levi et al., 1968) and pentobarbital (Held et al., 1970). A longer half-life was found in most patients with chronic liver disease for meprobamate (Held and von Oldershausen, 1969), chloramphenicol (Azzollini et al., 1972), rifampicin and isoniazid (Acocella et al., 1972), amobarbital (Mawer et al., 1972) and lidocaine (Thomson et al., 1973). Alternatively, Held et al. (1973) found that tolbutamide was eliminated more quickly in patients with acute hepatitis than in healthy persons. Furthermore, liver disease may also affect the plasma levels of compounds which are not primarily eliminated by metabolism in the liver, e.g. digoxin (Zilly et al., 1973c).

It seems evident from the literature data above cited that it is impossible to predict at present whether the elimination of a certain compound will be changed in a patient with liver disease. No correlations have been found between the common liver function tests (BSP retention, galactose elimination capacity, etc.) and drug metabolizing capacity of the liver. The liver content of cytochrome P-450, which is one of the most important drug metabolizing enzymes, may be determined after biopsy (Schoene et al., 1972). However, this is meaningful only as a measure of biotransformation capacity if the total liver volume is

known. At present it seems impossible to perform the determination of cytochrome P-450 and liver volume with acceptable accuracy. A complicating factor is that the enzyme may not be homogeneously distributed throughout the liver (Richter, 1974). Therefore, the estimation of the biological half-life of drugs which are completely metabolized in the human liver, has been applied in order to obtain information concerning an enhanced or decreased drug metabolism (Gillette, 1971). Hexobarbital has been used for such purposes in animal experiments, since the change in sleeping time may be related to the change of oxidative enzyme activity (Remmer, 1966). For a similar reason Richter et al. (1972) studied hexobarbital in patients with acute hepatitis and in healthy controls by measuring hexobarbital tolerance during i.v. infusion. Significantly less hexobarbital was required for the loss of counting ability in the patients when compared with the healthy subjects. On the basis of this finding, which at first instance would indicate an impaired hexobarbital metabolism in patients with impaired liver function, the present investigations on the pharmacokinetics of hexobarbital were initiated. In this Chapter pharmacokinetic data are presented for 38 patients with liver disease. These data should be compared to those obtained from healthy volunteers (Chapter 1, Section III). An attempt to correlate the results of hexobarbital pharmacokinetics with clinical and biochemical findings and common liver function tests will form the subject of further research. Ultimately, the utility and the predictive value of a hexobarbital test, for quantitative drug metabolism, may be evaluated.

METHODS

Hexobarbital sodium (EVIPAN) for i.v. injection was obtained from BAYER AG, Leverküsen, GFR. Solutions were prepared immediately before use by dissolving the sterile contents of an ampoule in sterile water.

All patients with liver disease, who participated in the present investigation, were hospitalized in the Medizinische Universitätsklinik at Würzburg, GFR (physicians in charge: Dr. W. Zilly and Dr. E. Richter). The patients gave their written consent to the experiments. Provisionally, they have been divided into two main subgroups: hepatitis and cirrhosis. Patients with hepatitis were diagnosed by histological examination after biopsy; cirrhotic patients by peritoneoscopy. Laboratory data, which were determined just prior to the experiments, and pathological findings are given in Table I and II. Prior to the experiments the patients had fasted overnight and they were resting in a supine position during the experiments. Hexobarbital sodium was intravenously administered by zero-order infusion. The infusion time and the administered dose are given

for each individual in Table I and II. Normally, infusion lasted 60 min, when 8 mg/kg of the sodium salt (equivalent to 7.32 mg/kg of the free acid) was administered. In some instances the drug was infused at a constant rate of 0.5 (mg/kg)/min until the loss of counting ability occurred. Blood samples (5 ml) were taken every 4 min during infusion, when the infusion time was shorter than 60 min. During a 60 min infusion, samples were taken at 10, 20, 30, 40, 50 and 60 min. After the termination of infusion, samples were taken at 10, 20, 30 min and 1, 2, 4, 6, 8, 10, 12, 18 and 24 h. The blood was centrifuged and the plasma was separated and frozen prior to assay. The hexobarbital plasma concentrations were determined by gas chromatography with nitrogen selective detection. Few times a purification step was necessary, which involved column chromatography, when the samples contained a high bilirubin content (Breimer and van Rossum, 1974; Chapter 2, Section II). A 48 - hour urine specimen was collected from some patients. Unchanged hexobarbital and 3-keto-hexobarbital were assayed as described in Chapter 2 and 3, respectively, Section II.

RESULTS

The hexobarbital plasma concentration curves after termination of the infusion were bi-exponential, with an initial rapid decline followed by a slower decay (Fig. 1 and 2). This kinetic behaviour is consistent with the two-compartment open model, which was also valid for hexobarbital kinetics in healthy subjects (Chapter 1, Section III). It suggests that hexobarbital distributes almost spontaneously into an initial dilution space (central compartment) consisting of blood and some well-perfused tissues, from which further distribution and elimination occur. The initial rapid decline of the plasma concentration is mainly due to distribution of the drug into less well-perfused tissues (peripheral compartment), whereas the subsequent more gradual slope is a reflection of the elimination of the drug by metabolism. The post-infusion plasma concentrations (C_p; mg/l) were fitted by the FARMFIT nonlinear regression analysis program (Chapter 1, Section I) according to the following equation (Chapter 1, Section III; Loo and Riegelman, 1970):

$$C_p = A_1 \cdot \frac{\tau_1}{T}(1 - e^{-T/\tau_1})\, e^{(T-t)/\tau_1} + A_2 \cdot \frac{\tau_2}{T}(1 - e^{-T/\tau_2})\, e^{(T-t)/\tau_2} \quad \text{(Eq. 1)}$$

where A_1 and A_2 are the coefficients (mg/l), T the infusion time (min) and τ_1 and τ_2 the time constants (min) of the rapid and the slower phase respectively.

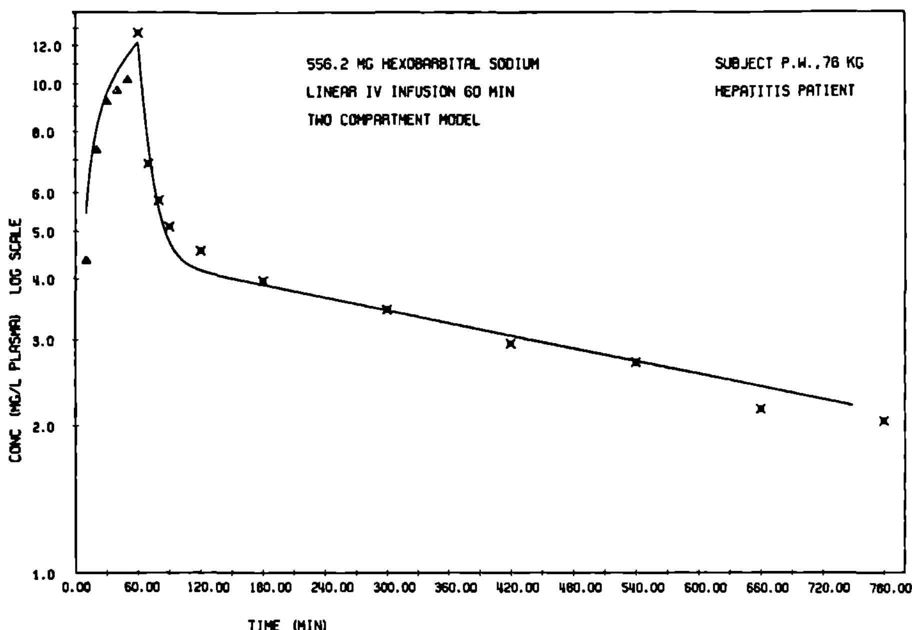

FIG. 1. Hexobarbital plasma concentration curve on semi-logarithmic scale during and after a 60 min zero-order i.v. infusion into a hepatitis patient (patient P.W., no. 3). The post-infusion concentrations were fitted according to Eq. 1. The curve during infusion was calculated on basis of the parameters of the post-infusion curve; the concentrations during infusion (triangles) have not been used in the fitting procedure. The reproduction was obtained from a direct computer plot. See Table I for the parameter values.

The initial graphical estimates of τ_1 and τ_2 were used as start guess for the fitting procedure. A relative error of 5% in the plasma concentrations was taken into account, since this was the standard deviation that was determined in the assay procedure (Chapter 2, Section II). Examples of fitted and directly plotted curves are given in Fig. 1 and 2. The most relevant parameters, intrinsic to the two-compartment open model, were calculated and these are given in Table I. Results for each patient are reported, since it was considered important to show the degree of variability in the patient population together with their clinical and biochemical data. The pharmacokinetic parameters of hexobarbital may be compared to those obtained for the healthy volunteers (Chapter 1, Section III). It may be argued that a comparison with a control group consisting of students between 20 and 25 years of age may not be valid, since the patient group is much more heterogeneous. Therefore additional

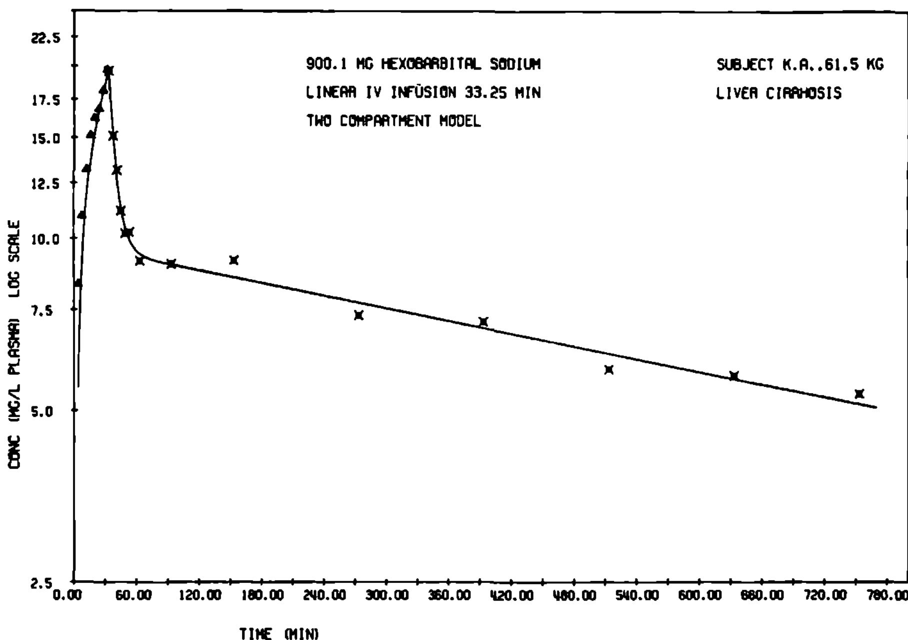

FIG. 2. Hexobarbital plasma concentration curve on semi-logarithmic scale during and after a 33.25 min zero-order i.v. infusion into a cirrhotic patient (patient K.A., no. 28). The post-infusion concentrations were fitted according to Eq. 1. The curve during infusion was calculated on basis of the parameters of the post-infusion curve; the concentrations during infusion (triangles) have not been used in the fitting procedure. The reproduction was obtained from a direct computer plot. See Table II for the parameter values.

TABLE III. Mean pharmacokinetic parameters of hexobarbital after i.v. infusion.

	n	$t_{1/2}$ (min)	k_{el}/kg (ml/min)	V_1/kg (l)	V_f/kg (l)
Hepatitis	18	483 ± 202*	2.12 ± 0.95*	0.35 ± 0.18	1.16 ± 0.38
Cirrhosis	20	834 ± 523*	1.48 ± 0.66*	0.47 ± 0.39	1.42 ± 0.71
Healthy	14	261 ± 69	3.57 ± 0.83	0.43 ± 0.16	1.10 ± 0.12

* Significantly different from healthy controls at $P < 0.001$ (Student's t-test).

experiments are being performed in healthy people of various ages and living circumstances, in order to allow more proper comparison.

In most hepatitis patients the hexobarbital plasma concentrations declined rapidly during the first hour after termination of infusion (mean τ_1, 22.5 min). Subsequently a slower phase followed, representing the elimination phase of the drug (mean τ_2, 690 min; mean half-life, 479 min). Compared to healthy controls the initial decay (τ_1) was similar in both groups, whereas the elimination half-life was more variable and prolonged for many hepatitis patients (Table I). A prolonged half-life was usually accompanied by a reduced metabolic clearance constant. Significant differences exist between the mean half-lives and clearance constants of the two groups (Table III). The average volume of the central compartment (V_1, 0.34 l/kg) and the average apparent volume of distribution (V_f, 1.16 l/kg) were almost the same for the two groups, but the individual values varied considerably among the diseased group. Comparable values were found for the distribution rate constants (r_{12}, 0.039 ± 0.025 and r_{21}, 0.018 ± 0.017).

The variation in the parameters of the patients with liver cirrhosis was even greater than in the hepatitis group (Table II). τ_1 varied from 4.4 tot 110 min and the elimination half-life from 261 tot 2285 min (mean $t_{1/2}$, 834 min), concomitantly with a reduced clearance constant. Significant differences exist between the mean half-lives and the clearance constants of the cirrhotic group and the healthy controls (Table III). The average distribution rate constants were not significantly different from those of the healthy group (r_{12}, 0.059 ± 0.046 and r_{21}, 0.019 ± 0.015).

For some patients the amount of unchanged drug and 3-keto-hexobarbital was determined in 48 hour's urine (Table IV). Compared to healthy controls (Chapter 1, Section III) it became evident that 3-keto-hexobarbital was excreted to a lesser extent in patients with liver desease. Urinary excretion of unchanged hexobarbital was mostly less than 1% of the dose administered.

→

Diagnosis and pathological findings:

1. acute hepatitis, prednisolone medication; 2. acute hepatitis; 3. acute hepatitis; 4. acute viral hepatitis, slight cholangitis; 5. acute viral hepatitis; 6. acute hepatitis; 7. acute viral hepatitis; 8. acute hepatitis, alcohol induced; 9. acute hepatitis, cholestasis; 10. acute hepatitis, cholestasis; 11. viral hepatitis, cholestasis; 12. viral hepatitis, cholestasis; 13. acute viral hepatitis, cholestasis; 14. hepatitis, cholestasis; 15. subacute viral hepatitis; 16. acute hepatitis; 17. subacute hepatitis; 18. no biopsy.

TABLE I. Hepatitis patients. Biochemical data and pharmacokinetic parameters of hexobarbital.

	Age (yr)	Body-weight (kg)	Total Bilirubin (mg/100 ml)	SGOT (mU/ml)	SGPT (mU/ml)	Albumin (g/100 ml)	γ-Glob (g/100 ml)	Dose/kg (mg)	Inf. time (min)	τ_1 (min)	τ_2 (min)	$t_{1/2}$ (min)	V_1/kg (l)	V_f/kg (l)	k_{el}/kg ml/(min)
1. R.L.	46	98	10.1	312	420			2.97	6.5	13.9	604	419	0.17	0.83	1.53
2. H.H.	28	68	7.9	240	444			4.12	9.0	19.4	542	376	0.29	0.54	1.04
3. P.W.	60	76	2.7	300	640	4.1	1.9	7.32	60.0	11.0	1004	697	0.16	1.34	1.47
4. B.E.	37	63	8.7	840	1080	5.1	0.8	7.32	60.0	8.3	1288	894	0.12	1.00	0.82
5. T.C.	23	65	4.8	640	1200	4.6	0.9	7.32	60.0	29.7	557	387	0.29	1.06	2.34
6. D.H.	23	80	6.9	360	1600	4.4	1.7	7.32	60.0	32.3	673	467	0.25	0.82	1.40
7. V.B.	21	73	8.3	900	2100	4.5	1.6	7.32	60.0	15.4	602	418	0.23	1.35	2.62
8. B.H.	33	76	7.3	536	564			2.97	6.5	9.7	370	257	0.21	1.13	3.52
9. M.C.	19	57	6.4	280	690	3.6	1.3	7.32	60.0	17.0	734	510	0.35	1.19	1.73
10. K.W.	26	65	7.1	42	300	4.2	1.3	7.32	60.0	70.4	486	338	0.55	1.02	2.57
11. S.L.	52	69	20.1	112	96			4.80	10.5	20.2	656	456	0.61	2.21	3.71
12. K.T.	21	65	11.0	28	60	2.9	2.0	7.32	60.0	14.2	486	338	0.34	1.02	2.25
13. S.V.	62	73	9.3	468	216			5.72	12.5	15.4	1124	781	0.68	1.05	0.94
14. G.P.	75	75	8.5	880	1920	3.9	2.1	7.32	60.0	32.6	1315	913	0.43	1.50	1.23
15. K.H.	17	69	1.9	42	52	4.2	1.8	7.32	60.0	36.0	713	495	0.46	1.53	2.51
16. L.A.	17	47	3.7	92	320	4.0	1.7	7.32	60.0	24.4	417	290	0.20	0.82	2.75
17. F.E.	24	78.5	6.6	60	440	4.5	1.1	7.32	60.0	21.6	346	240	0.35	0.86	2.80
18. S.O.	58	67	5.9	70	228	4.1	1.1	7.32	60.0	14.1	503	349	0.26	1.54	3.67
Mean	35.7	70.3	7.6	345	687	4.2	1.5	6.43	45.8	22.5	690	479	0.34	1.16	2.16
SD.	18.4	10.6	3.9	302	634	0.5	0.4	1.59	23.5	14.5	298	207	0.16	0.38	0.93

TABLE II. Patients with liver cirrhosis. Biochemical data and pharmacokinetic parameters of hexobarbital.

	Age (yr)	Body-weight (kg)	Total Bilirubin (mg/100 ml)	SGOT (mU/ml)	SGPT (mU/ml)	Albumin (g/100 ml)	γ-Glob (g/100 ml)	Dose/kg (mg)	Inf. time (min)	τ_1 (min)	τ_2 (min)	$t_{1/2}$ (min)	V_1/kg (l)	V_f/kg (l)	k_{el}/kg ml/(min)
19. S.K.	45	58	1.9	17	17	3.9	1.3	10.75	23.5	4.4	376	261	0.26	1.15	3.20
20. D.F.	39	82	0.2	10	6	5.0	1.0	6.75	14.75	42.3	528	367	0.31	0.95	2.38
21. K.F.	44	103	0.8	24	24	4.0	1.1	4.57	10.0	11.9	672	467	0.20	1.05	1.71
22. M.H.	48	72	0.9	29	17	4.8	1.1	5.72	12.5	15.2	609	423	0.22	0.87	1.56
23. G.H.	36	83	1.0	13	7	4.2	1.3	6.97	15.25	7.2	845	587	0.17	0.89	1.09
24. N.E.	47	63	0.9	28	19	3.9	1.5	9.60	21.0	35.6	948	658	0.55	1.22	1.36
25. B.A.	43	92.5	0.8	16	10	3.5	1.8	5.49	12.0	11.9	1171	813	0.23	1.57	1.43
26. B.G.	52	67.5	1.8	30	18	2.3	2.8	7.32	60.0	11.1	692	481	0.34	0.99	1.47
27. B.F.	51	76.5	6.8	140	88	2.6	2.5	7.32	60.0	49.0	1296	900	0.83	1.36	1.08
28. K.A.	35	68	2.8	40	36	3.6	1.7	15.20	33.25	7.3	1175	816	0.27	1.49	1.31
29. R.E.	38	80	1.7	24	20	4.0	1.3	7.32	60.0	28.8	821	570	0.20	0.69	0.94
30. H.E.	44	73	2.8	22	28	3.3	3.1	7.32	60.0	14.6	1150	799	0.24	1.15	1.06
31. S.F.	62	67.5	3.0	26	14	2.0	5.5	7.32	63.5	110	2924	2031	1.37	1.78	0.62
32. M.P.	73	75	6.6	34	14	2.4	3.3	7.32	60.0	6.6	3290	2285	0.31	1.92	0.59
33. K.R.	38	75	2.0	300	130	3.3	4.4	7.32	60.0	9.9	1624	1128	0.24	1.25	0.79
34. Z.A.	35	54.5	12.6	60	70	3.4	1.2	7.32	60.0	22.6	1890	1313	1.15	4.12	2.25
35. E.A.	46	82	8.4	180	132	3.4	2.2	3.09	6.75	11.8	1363	947	0.22	1.38	1.06
36. K.W.	55	84.5	1.3	30	22	4.7	2.0	7.32	60.0	21.1	708	492	0.35	1.45	2.30
37. K.H.	45	66	2.0	74	60	3.3	2.3	7.32	60.0	87.3	1085	753	1.35	1.72	1.87
38. G.R.	45	84	1.2	36	8	2.5	2.6	5.49	12.0	4.6	859	597	0.67	1.32	1.55
Mean	46.0	75	3.0	57	37	3.5	2.2	7.34	38.2	25.7	1201	834	0.47	1.42	1.48
S.D.	9.6	12	3.2	72	39	0.8	1.2	2.45	23.3	28.2	752	523	0.39	0.71	0.66

Diagnosis and pathological findings:

19. cirrhosis, ascites, developing portal hypertension, prednisolone medication; 20. cirrhosis; 21. cirrhosis; 22. cirrhosis; 23. alcoholic cirrhosis, developing portal hypertension; 24. cirrhosis, cholestasis; 25. cirrhosis, portal hypertension; 26. cirrhosis, ascites, moderate portal hypertension, diabetes mellitus; 27. cirrhosis, portal hypertension, oesophageal varices; 28. progressive cirrhosis, portal hypertension, prednisolone medication; 29. post-necrotic cirrhosis portal hypertension, cholestasis, ascites; 30. cirrhosis, portal hypertension, oesophageal varices; 31. progressive cirrhosis, portal hypertension, ascites; 32. cirrhosis with jaundice, ascites; 33. cirrhosis, moderate portal hypertension, severe fatty degeneration; 34. alcoholic cirrhosis, jaundice, ascites; 35. cirrhosis, (patient died, necropsy revealed severe intrahepatic cholestasis); 36. alcoholic cirrhosis (patient died from oesophageal varices bleeding); 37. cirrhosis, pancreatitis, diabetes mellitus; 38. cirrhosis, portal hypertension, oesophageal varices, ascites (patient died after shunt operation).

←

TABLE III. Cumulative urinary excretion of hexobarbital and 3-keto-hexobarbital

Patients	Hexobarbital (% of dose administered)	3-Keto-hexobarbital (% of dose administered)
Hepatitis		
P.W.	0.58	7.1
T.C.	0.12	16.3
G.P.	0.31	2.8
L.A.	0.15	18.3
S.O.	0.14	13.8
Cirrhosis		
G.H.	0.18	33.2
B.G.	0.19	21.2
M.P.	1.10	5.1
K.H.	0.30	4.7

DISCUSSION

The metabolic clearance of hexobarbital

It is evident that the rate of elimination of hexobarbital is retarded in many patients with liver disease; particularly in cases with chronic liver disease (cirrhosis) the half-life may be extremely long. It was outlined in Chapter 1, Section I, that the half-life of a drug is mainly dependent on its renal or metabolic clearance and on the apparent volume of distribution. The actual

elimination process of a drug from the body is characterized by the clearance constant (k_{el}; ml/min). It is a measure of the functional ability of a substance to be removed by the eliminating organ (Riegelman and Rowland, 1973). In the majority of previous studies, concerning drug metabolism associated with impaired liver function, only the half-life of the drug was measured. The possibility exists that essential differences in metabolic clearance are obscured by simultaneously occurring alterations in the apparent volume of distribution. Therefore, in the present investigation with hexobarbital, it is focussed on the metabolic clearance constant which is significantly decreased for the patients with liver disease. The clearance constant depends on the enzymatic constants governing biotransformation (Chapter 1, Section I):

$$k_{el} = \frac{\dot{Q}_m}{K_m + C} \quad \text{(Eq. 2)}$$

where $\dot{Q}_m$ is the capacity of the liver enzymes involved (mg/min), which is related to V_{max} when only one enzyme is involved. K_m is the apparent Michaelis-Menten constant (mg/l) and C the drug concentration in the clearance tissue (mg/l). At low plasma concentrations ($C \ll K_m$) k_{el} is often a simple function of the enzymatic constants ($k_{el} = \dot{Q}_m/K_m$). If it is assumed that K_m has not changed in the diseased situation, it may be concluded that a reduced clearance is caused by a decreased enzymatic capacity of the liver. For hexobarbital this may be due, for instance, to a reduced cytochrome P-450 content of the liver, which was shown in cases of severe hepatitis and liver cirrhosis (Schoene et al., 1972; von Oldershausen et al., 1973; Zilly et al., 1973b). However, it cannot be excluded that the affinity of the enzyme system for a certain drug (K_m) is not influenced by liver disease. Conformational changes in the enzyme system or alterations in the microenvironment of the enzymes may be induced by the presence of abnormal amounts of endogenous substances, e.g. bilirubin during acute hepatitis, or bile acids during cholestasis (Gillette et al., 1957; McLuen and Fouts, 1961). An increased K_m-value will reduce the metabolic clearance of a drug. A more complicated situation arises if the drug concentration in the liver becomes critical with respect to K_m. The clearance becomes concentration dependent and apparent saturation of the enzyme system may be encountered. An example of such a situation is given in Fig. 3 (upper), where the elimination rate shortly after infusion is slower than that at lower concentrations after longer periods. This may be due either to a reduced K_m-value or to an increased concentration of drug at the biotransformation site. Such a phenomenon was observed only in few patients.

As well as on the enzyme constants, metabolic clearance may also be dependent

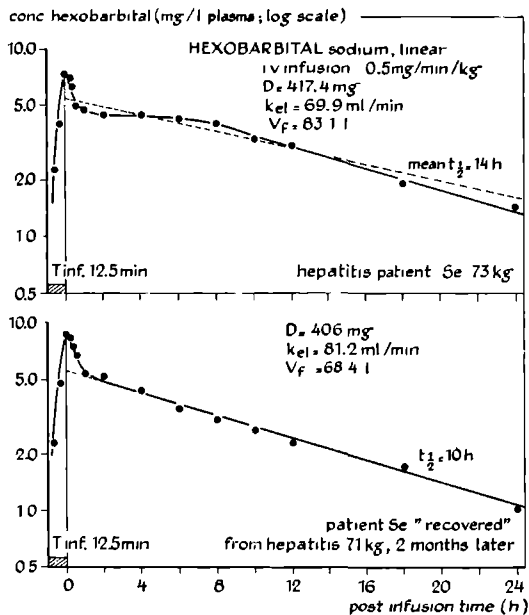

FIG. 3. *Upper*: Hexobarbital plasma concentration curve on semi-logarithmic scale during and after a 12.5 min zero-order infusion into a hepatitis patient. The shape of the post-infusion curve suggests that elimination may be capacity-limited. Some parameters were estimated, assuming an average half-life of 14 h. *Lower*: The experiment was repeated after 2 months, when the patient had recovered, as judged by clinical symptoms and laboratory data. It may be noted that the shape of the curve had returned to normal; however, the metabolic clearance constant was still very much reduced and the half-life prolonged.

on the blood flow through the liver (Nagashima and Levy, 1968; Rowland et al., 1973). This becomes an important factor if the clearance for a certain drug is in the same order of magnitude as the blood flow through the liver (normally about 1500 ml/min). It was shown previously that lidocaine clearance, which is 700 - 800 ml/min in healthy persons (Rowland et al., 1971), was significantly reduced in patients with reduced cardiac output (Thomson et al., 1973). For hexobarbital, blood flow probably is a less critical factor, since its clearance is relatively small compared to blood flow. It is possible however, that with liver disease, alterations in the regional perfusion pattern may substantially contribute to a less effective extraction of the drug by the liver. This leads to an apparent reduction of drug metabolizing capacity.

Obviously, there are several theories which may explain the reduced hexo-

barbital clearance in patients with liver disease. Which of these is valid, is difficult to state since they may vary from patient to patient. All the mentioned processes are possibly involved to some extent, although the decreased amount of cytochrome P-450 in the liver seems one of the most important explanations (von Oldershausen et al., 1973). The reason that some patients, in spite of severe clinical symptoms, showed a quite normal clearance value, may be attributed to drug history of these patients. Levi et al. (1968) found that the phenylbutazone half-life was prolonged in those patients with liver disease, who had not received any previous drug treatment. On the other hand, patients who had received a variety of drugs known to induce microsomal enzymes, showed a quite normal half-life in the diseased state. These authors suggest that previous investigators may have failed to demonstrate the impairment of drug metabolism in patients with hepatic dysfunction, because the importance of other drug treatment was not taken into account. It is possible that in the present investigations with hexobarbital, drug treatment prior to hospitalization is a factor that should be considered. It may explain the relatively short half-life in some patients. The influence of previous drug treatment on the pharmacokinetics of hexobarbital will be the subject of a further study.

The hexobarbital experiment was repeated, for some patients, when clinical symptoms and biochemical data suggested apparent recovery from the liver disease. In Fig. 3 an example is given for such a patient who received the hexobarbital infusion with an interval of 2 months. The metabolic clearance was still much reduced on the second occasion, although the kinetic behaviour of hexobarbital was no longer capacity-limited as it was at the first trial. It was also found for other patients that apparent clinical recovery is not accompanied by a normalization of the metabolic capacity of the liver, as measured by the hexobarbital clearance.

Hexobarbital tolerance and the volume of the central compartment

It was shown previously that patients with acute hepatitis exhibited a reduced tolerance to the CNS depressant effect of hexobarbital (Richter et al., 1972; Zilly et al., 1973b). This tolerance was defined as the amount of drug (mg/kg) required to cause loss of counting ability during a constant-rate infusion. In these patients the plasma levels during infusion increased faster than in healthy controls, so that at an earlier stage the effective concentration was reached (Zilly et al., 1973a). The cytochrome P-450 content of the liver in patients with acute hepatitis was significantly reduced (Zilly et al., 1973b). For this reason, initially a correlation was supposed to exist between hexobarbital

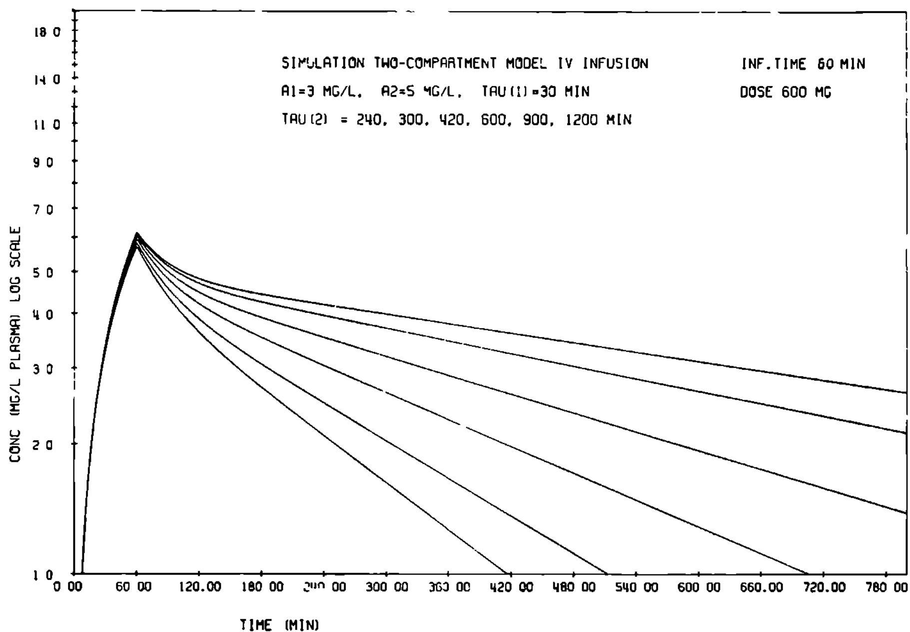

FIG. 4. Simulation of the influence of τ_2 on the plasma concentration curve during infusion. It may be noted that despite a great variation of τ_2, the influence on the rise of the concentration during infusion is almost negligible.

tolerance and the metabolic capacity of the liver. From the kinetic experiments with hexobarbital it became evident that the metabolic clearance is generally reduced in these patients, causing a longer elimination half-life. In Fig. 4 the influence of the elimination rate on the concentration time course during a 60 min infusion was simulated. It is evident that the influence of the elimination half-life on the rise of plasma concentration during a relatively short infusion period, is almost negligible. The responsible factor appears to be the volume of the central compartment (V_1). This is smaller in many patients with acute hepatitis (Table I, patients 1 - 8) and in Fig. 5 the coefficient A_1 has been varied, which is greatly determined by V_1. It can be observed that this parameter greatly influences the plasma concentration during infusion. The physiological meaning of a reduced volume of the central compartment is uncertain, although it is likely that the liver is involved. The liver may not belong to the central compartment, which means that the drug does not instantaneously distribute into the liver. On the other hand, for a lipophilic drug such as hexobarbital,

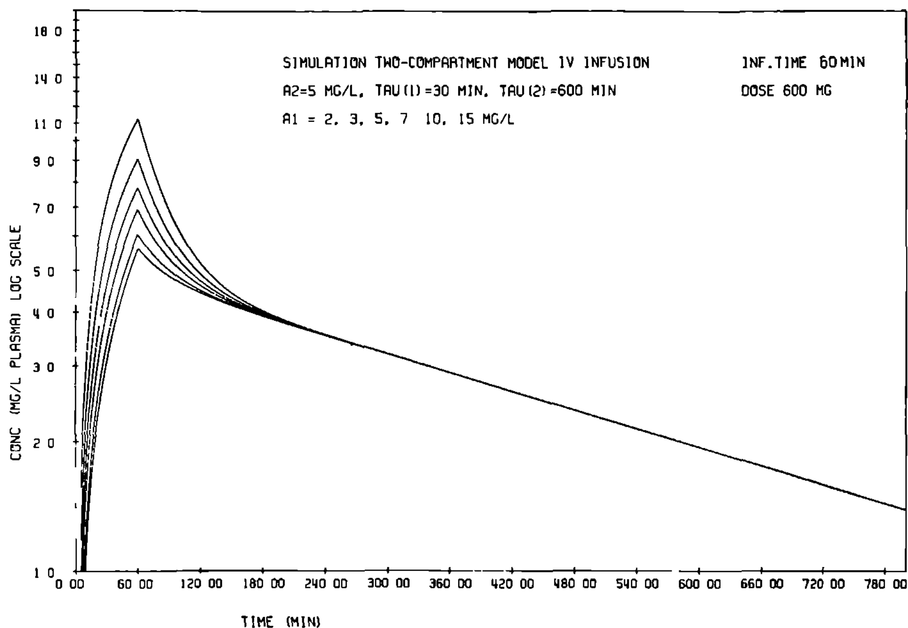

FIG. 5. Simulation of the influence of A_1 (which is greatly determined by the volume of the central compartment V_1) on the plasma concentration curve during infusion. It may be noted that the rise of the plasma concentration varies substantially with A_1.

the brain is part of the central compartment. If V_1 is reduced, the brain concentration rises more rapidly and the central tolerance is found reduced.

The apparent volume of distribution

The distribution of a drug outside the plasma is characterized by the apparent volume of distribution (V_f). This parameter is sometimes referred to by „volume at steady state", which indicates that it is the volume of distribution when equilibrium between plasma and tissues has been reached, as derived from the plasma concentration. As outlined in Chapter 1, Section I, the half-life of a drug is also greatly dependent on the volume of distribution. For hexobarbital this volume is more than 1 l/kg body-weight, which indicates extensive tissue localization of the drug once it has been distributed. The apparent increase in distribution volume for some patients, particularly with liver cir-

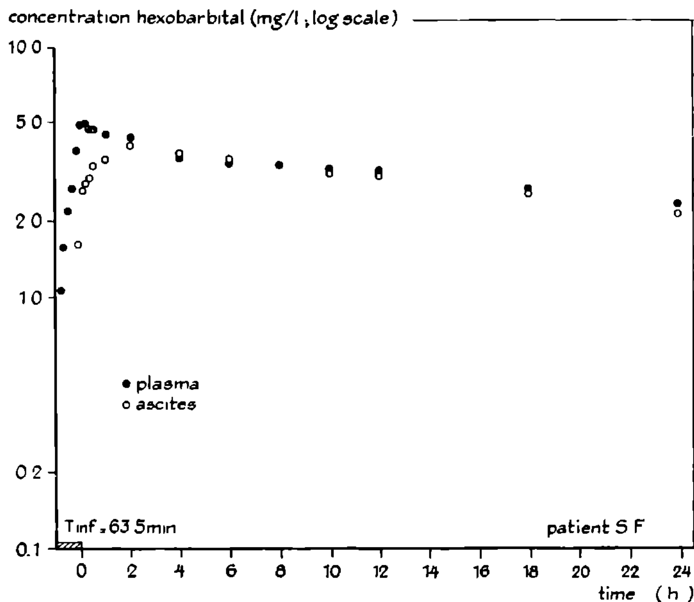

FIG. 6. Concentration of hexobarbital in plasma and in ascitic fluid (semi-logarithmic scale) of a patient with liver cirrhosis (patient S.F., no. 31) after a 63.5 min zero-order i.v. infusion. Two hours after the termination of the infusion the concentration in the plasma and in the ascitic fluid have become almost identical. The elimination half-life was approximately 34 h, k_{el} = 41.9 ml/min and V_f = 120 l.

rhosis, is comparable to similar findings for lidocaine in patients with chronic liver disease (Thomson et al., 1973). Alterations in plasma protein binding or tissue binding due to the diseased state may be contributing factors (Reidenberg and Affirme, 1973). However, in the present investigation no correlation was found between the plasma albumin content and the apparent volume of distribution. Protein binding of hexobarbital to 5% albumin at 100 mg/l is approximately 56% (Richter, 1974). An extensive study with pentobarbital revealed that binding of pentobarbital to plasma protein (about 70% at 50 mg/l) in several patients with liver disease was comparable to healthy controls (Fuchshofen, 1974). These findings suggest that plasma protein binding is a factor of limited importance with respect to the volume of distribution of hexobarbital. Some of the cirrhotic patients with an enlarged volume of distribution for hexobarbital also exhibited ascites (Table II). An additional experiment revealed that the hexobarbital concentration in the ascitic fluid was equal to the plasma concentration shortly after the termination of infusion

(Fig. 6; patient S.F., no. 31). Since the volume of this fluid probably did not exceed 10 l for this patient, the preliminary conclusion may be drawn that accumulation of hexobarbital in ascitic fluid does not contribute substantially to the enlarged volume of distribution. It is likely that other factors are involved and these will require further investigation.

Concluding remarks

The preliminary results, on the pharmacokinetics of hexobarbital in patients with liver disease, indicate that several changes in the kinetic behaviour of hexobarbital may occur when compared to healthy persons. A reduced metabolic clearance, resulting in a prolonged half-life, is the most evident finding. Furthermore, alterations in the volume of the central compartment or the apparent volume of distribution are apparent in some patients. It will be the subject of further research to subdivide the patients who participated in the present study, according to more detailed clinical and biochemical data and an attempt will be made to find correlations between certain pharmacokinetic parameters and biochemical data or common liver function tests.

SUMMARY

The pharmacokinetics of hexobarbital were studied in 18 patients with hepatitis and 20 patients with liver cirrhosis. Hexobarbital sodium was administered by zero-order i.v. infusion and plasma levels were determined at regular intervals during and after infusion.

The kinetic behaviour of hexobarbital after infusion was consistent with the two-compartment open model. Plasma concentrations were fitted according to the post-infusion equation and the pharmacokinetic parameters were estimated. These have been compared with the results obtained for healthy volunteers (Chapter 1, Section III). The elimination half-life was considerably prolonged for most patients; hepatitis: 257 - 913 min, mean 479 min; cirrhosis: 261 - 2285 min, mean 834 min. The metabolic clearance constants were found to be significantly reduced, due to a decreased metabolic capacity of the liver. It was discussed also that changes in the apparent Michaelis-Menten constant may be involved, which in some instances lead to an apparent saturation of the metabolizing enzymes. The average distribution rate constants were found to be comparable to healthy controls. The metabolite 3-keto-hexobarbital was excreted to a lesser extent into the urine.

It was demonstrated that the previously reported reduced central tolerance of patients with acute hepatitis to hexobarbital during i.v. infusion, was due to a decreased volume of the central compartment. Plasma concentrations during infusion rise more rapidly in such patients. Simulation experiments revealed that the

rate of drug elimination does not influence drug concentrations during infusion to any significant extent.

The average apparent volume of distribution of the patients with liver disease was comparable to that of healthy controls. For some cirrhotic patients, however, this volume was considerably enlarged. An additional experiment showed that the hexobarbital concentrations in ascitic fluid were equal to the plasma concentration, which suggests that this fluid does not contribute substantially to an enlarged distribution volume.

It was concluded that several changes in the kinetic behaviour of hexobarbital in patients with liver disease may become apparent. The underlying causes of their changes will require further investigation.

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Douwe Durk Breimer werd geboren op 24 november 1943 te Oudemirdum (Gaasterland). Hij bezocht van 1956 - 1960 de Chr. Mulo-school te Balk (diploma Mulo-B) en van 1960 - 1962 het Bogerman Lyceum te Sneek (diploma H.B.S.-B). Hij studeerde van 1962 - 1970 farmacie aan de Rijksuniversiteit te Groningen; het kandidaatsexamen werd in 1965 cum laude afgelegd. In het kader van het gekozen bijvak farmaceutische chemie verrichtte hij in 1967 gedurende 3 maanden onderzoek aan de University of Strathclyde te Glasgow, Schotland, onder leiding van Prof. J. B. Stenlake. Het doctoraalexamen werd in 1969 cum laude afgelegd, terwijl hij in oktober van hetzelfde jaar de K.N.M.P.-prijs ontving. Het apothekersexamen vond in juli 1970 plaats.

In augustus 1970 trad hij in dienst van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek te 's-Gravenhage en vanaf die tijd is hij werkzaam op het Farmacologisch Laboratorium te Nijmegen. Hij heeft als lid van de werkgroep farmacokinetiek onder leiding van Prof. Dr. J. M. van Rossum onderzoek verricht naar farmacokinetische en biofarmaceutische aspekten van slaapmiddelen, voornamelijk bij de mens. Daarnaast werd tezamen met Dr. T. B. Vree, Drs. C. A. M. van Ginneken en Prof. Dr. J. M. van Rossum gewerkt aan de analyse van cannabinoiden in hashish en marihuana, met behulp van gaschromatografie en gecombineerde gaschromatografie - massaspectrometrie. Sedert eind 1972 wordt samengewerkt met Dr. E. Richter en Dr. W. Zilly, Medizinische Universitätsklinik, Würzburg, West-Duitsland, in een projekt betreffende de farmacokinetiek van geneesmiddelen bij patiënten met leverziekte.

Hij is lid van het Bestuur van de sectie Farmacochemie van de Koninklijke Nederlandse Chemische Vereniging en lid van de Centrale Commissie voor Studiezaken van de Koninklijke Nederlandse Maatschappij ter bevordering der Pharmacie.

Hij is gehuwd en heeft drie dochters.

Uit onderzoekingen waaraan is meegewerkt, zijn de volgende publikaties voortgekomen (chronologische volgorde):

1. J. B. Stenlake, D. D. Breimer, G. A. Smail, A. Stafford and W.C. Bowman: The preparation and properties of (+)- and (—)-guanoxan. *J. Pharm. Pharmacol. 20,* Suppl., 82S - 93S (1968).

2. J. M. van Rossum, D. D. Breimer, C. A. M. van Ginneken, J. M. G. van Kordelaar and T. B. Vree: Gas-liquid chromatography in pharmacology and toxicology; pharmacokinetic analysis limited by the sensitivity of the analytical technique. *Clin. Chim. Acta 34,* 311 - 319 (1971).

3. T. B. Vree, D. D. Breimer, C. A. M. van Ginneken, J. M. van Rossum, R. A. de Zeeuw and A. H. Witte: Identification of cannabivarins in hashish by a new method in combined gas chromatography - mass spectrometry. *Clin. Chim. Acta 34,* 365 - 370 (1971).

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5. T. B. Vree, D. D. Breimer, C. A. M. van Ginneken and J. M. van Rossum: Identification of the methyl and propyl homologues of CBD, THC and CBN in hashish by a new method of combined GC-MS. *Acta Pharmaceut. Suec. 8,* 671 - 672 (1972).

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8. D. D. Breimer, T. B. Vree, C. A. M. van Ginneken and J. M. van Rossum: Some new Cannabis constituents. In: *Biochemical and Pharmacological aspects of dependence and reports on marihuana research,* p. 116 - 118. H. M. van Praag, Ed., Erven Bohn B.V., Haarlem (1972).

9. D. D. Breimer, T. B. Vree, C. A. M. van Ginneken, P. Th. Henderson and J. M. van Rossum: Identification of Cannabis metabolites by a new method of combined gas chromatography - mass spectrometry. *Proceedings of the International Symposion on gas chromatography - mass spectrometry,* p. 88 - 107, A. Frigerio, Ed., Tamburini Editore, Milano (1972).

10. C. A. M. van Ginneken, T. B. Vree, D. D. Breimer, H. H. W. Thijssen and J. M. van Rossum: Cannabinodiol, a new hashish constituent identified by gas chromatography - mass spectrometry. *Proceedings of the International Symposion on gas chromatography - mass spectrometry,* p. 110-129, A. Frigerio, Ed., Tamburini Editore, Milano (1972).

11. R. A. de Zeeuw, J. Wijsbeek, D. D. Breimer, T. B. Vree, C. A. M. van Ginneken and J. M. van Rossum: Cannabinoids with a propyl side chain in Cannabis;

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18. D. D. Breimer and J. M. van Rossum: Pharmacokinetics of (+)-, (—)- and (±)-hexobarbital in man after oral administration. *J. Pharm. Pharmacol. 25,* 762 - 764 (1973).

19. D. D. Breimer, H. L. M. Cox and J. M. van Rossum: Relative bioavailability of chloral hydrate after rectal administration of different dosage forms. *Pharm. Weekblad 108,* 1101 - 1110 (1973).

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22. D. D. Breimer and J. M. van Rossum: Pharmacokinetics of the enantiomers of hexobarbital studied in the same rat and in the same isolated perfused rat liver. *Eur. J. Pharmacol. 26,* 321 - 330 (1974).

23. D. D. Breimer, C. Honhoff, W. Zilly, E. Richter and J. M. van Rossum: Pharmacokinetics of hexobarbital in man after intravenous infusion. Submitted for publication.

24. W. Zilly, D. D. Breimer and E. Richter: The influence of rifampicin on the pharmacokinetics of hexobarbital and tolbutamide in healthy volunteers. Submitted for publication.

STELLINGEN

I

Farmacokinetische en biofarmaceutische overwegingen behoren een belangrijke rol te spelen bij de keuze van een slaapmiddel. Daarbij verdienen in het algemeen stoffen met een vrij korte halfwaardetijd de voorkeur, terwijl de te verkiezen toedieningsvorm moet zijn afgestemd op de snelheid waarmee het effekt gewenst wordt.

Dit proefschrift.

II

De gebruikelijke indeling van de barbituraten naar werkingsduur bij de mens is misleidend, mede omdat deze berust op onjuiste veronderstellingen betreffende de snelheid waarmee zij worden geïnaktiveerd.

Martindale (1972), *The Extra Pharmacopoeia*, 26th ed., p. 891.
Mark, L. C. (1969), *Clin. Pharmacol. Ther. 10*, 287.
Dit proefschrift.

III

In onderzoekingen bij ratten, waarbij de verlenging of verkorting van de slaaptijd na toediening van hexobarbital geldt als maat voor verandering in de oxydatieve enzymaktiviteit, verdient het de voorkeur alleen de rechtsdraaiende isomeer van hexobarbital te gebruiken.

McCarthy, J. S. en R. E. Stitzel (1971), *J. Pharmacol. Exp. Ther. 176*, 772.
Dit proefschrift.

IV

De toediening van 5 gram DDT ter behandeling van patiënten met barbituraat-intoxicaties, met de bedoeling een snelle induktie van de metaboliserende enzymen te bewerkstelligen, is niet rationeel en moet als onverantwoord worden beschouwd.

Rappolt, R. T. (1973), *Clin. Toxicol. 6*, 147.

V

Voor de bepaling van Δ^1-tetrahydrocannabinol in plasma verdient de methode van Agurell et al. (1973), welke gebruik maakt van massafragmentografie, de voorkeur boven de gaschromatografische methode van Garrett en Hunt (1973).

Agurell, S. et al. (1973), *J. Pharm. Pharmacol. 25*, 554.
Garrett, E. R. en C. A. Hunt (1973), *J. Pharm. Sci. 26*, 1211.
Breimer, D. D. (1973), *Pharm. Weekblad 108*, 1017.

VI

De opvatting betreffende het bestaan van een funktionele relatie tussen de regionale verdeling van monoaminen binnen de hersenen en het slaap - waak mechanisme wordt niet gesteund door recent onderzoek.

Jouvet, M. (1969), *Science 163*, 32.
Jalowiec, J. E. et al. (1973), *Exp. Neurol. 41*, 670.

VII

De berekening van farmacokinetische parameters uit gemiddelde plasmacurves kan tot onjuiste konklusies leiden. Bij voorkeur dienen de parameters berekend te worden per individu, welke vervolgens eventueel statistisch kunnen worden bewerkt.

VIII

Het verdient aanbeveling voorschriften voor die bereidingen van geneesmiddeltoedieningsvormen in de Nederlandse Farmacopee op te nemen, welke uit farmacotherapeutisch oogpunt zinvol zijn en biofarmaceutisch voldoende zijn onderzocht. De eis dat de apotheker „op de juiste wijze en nauwkeurig volgens het recept bereidt"[1]), behoort een waarborg in te houden voor het afleveren van betrouwbare toedieningsvormen.

[1]) Besluit uitoefening artsenijbereidkunst art. 19, lid 1.

IX

Nu de drinkwaterfluoridering op de meeste plaatsen in Nederland is opgeschort, dienen ter noodzakelijke vervanging daarvan zodanige fluoride bevattende toedieningsvormen te worden ontwikkeld, dat zoveel mogelijk het lokale kontakt van fluoride met het gebit wordt bevorderd.

X

De ruime aandacht die recent door de publiciteitsmedia is besteed aan de mogelijk nadelige aspekten van geneesmiddelen, heeft in sterke mate de noodzaak vergroot patiënten die noodzakelijk gedurende korte of langere tijd een geneesmiddel dienen te gebruiken te motiveren zich nauwkeurig aan het voorgeschrevene te houden.

XI

Aangezien adoptie vóór alles in het belang van het kind moet zijn, verdient het aanbeveling art. 228 sub c, boek 1 van het burgerlijk wetboek, voor zover daarin vermeld dat ieder der adoptanten ten hoogste 50 jaar ouder dan het kind mag zijn, zodanig worden gewijzigd dat het maximum leeftijdsverschil wordt gesteld op 35 jaar. De richtlijnen inzake opname van buitenlandse adoptiefkinderen dienen overeenkomstig te worden aangepast.

XII

De suggestie van staatssecretaris Veerman om de eindexamens in het voortgezet onderwijs in tijd en niveau te spreiden, is voor scholen die werken met een systeem van jaarklassen om redenen van school- en onderwijsorganisatie niet funktioneel.

XIII

Vrouwenemancipatie betekent mannenparticipatie.

XIV

Een goed gefundeerde stelling staat op vier poten.

D. D. BREIMER, Nijmegen, 14 juni 1974.