pharmacokinetics of antipyretic and anti-inflammatory analgesics
PHARMACOKINETICS OF ANTIPYRETIC AND
ANTI-INFLAMMATORY ANALGESICS

a fundamental kinetic study in man and its
clinical-pharmacological implications
Promotor: Prof. Dr. J. M. van Rossum.
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PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WISKUNDE EN NATUURWETENSCHAPPEN AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF. DR. A.J.H. VENDRIK VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN HET OPENBAAR TE VERDEEDIGEN OP WOENSDAG 8 SEPTEMBER 1976 DES NAMIDDAGS TE 4.00 UUR

DOOR

CORNELIS ANTONIUS MARIA VAN GINNEKEN

GEBOREN TE ETTEN-LEUR

1976

DRUK: STICHTING STUDENTENPERS NIJMEGEN
The investigations were carried out in the Department of Pharmacology, University of Nijmegen, Nijmegen, The Netherlands, and were supported in part by grants from the Dutch Foundation for Medical Research (Fungo-ZWO) and from the Prevention Fund of the Dutch Ministry of Health.
aan mijn ouders
voor Cécile, Hubrecht en Ewout
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In this thesis a number of pharmacokinetic investigations in man are described. The compounds that were administered are antipyretic and anti-inflammatory analgesics. Within this widely used class of drugs it appeared possible to find a combination of practical and theoretical interest.

For all drugs studied the pharmacokinetic results are discussed on basis of the separate processes that constitute the overall kinetic pattern. The thesis is divided into three main sections:

- the first section contains a general introduction into the field as well as some methods that were used throughout the investigations
- in the second section linear pharmacokinetics are discussed. For most of the drugs in this study the kinetic behaviour can be described adequately on basis of simple models. Attention is paid to the mechanisms by which the drugs are eliminated from the body. The renal clearance is discussed in some detail, although this quantitatively is of minor importance
- the third section is devoted to non-linear pharmacokinetics. Methods are proposed to calculate the relevant parameters in case of capacity-limited elimination. The significance of non-linear pharmacokinetic behaviour of drugs is discussed, also when these drugs are administered in a chronic dosage regimen, with special reference to salicylates.

This thesis gives the answer to some questions, but much more questions arise from it. A lot of intriguing problems obtruded upon us during the investigations and one of the major results of our study is the fact that background information is supplied for a lot of forthcoming research.
GLOSSARY

t  time after administration of a drug (min or hr)
D  dose of drug administered (e.g. mg)
F  fraction of the administered dose, that reaches the general circulation intact (biological availability)
C  concentration of drug (mg/l), usually in plasma; if the concentration in another compartment is referred to, this is indicated with an appropriate suffix
C_{pl}  plasma concentration at any time during a dosage interval in a plateau situation
\bar{C}_{pl}  average plateau concentration in plasma
V  volume of distribution, assuming a one-compartment pharmacokinetic model; in case of more compartments a suffix denotes which compartment is referred to (e.g. 1)
t_{1/2a}  half-life of absorption, assuming a first-order absorption process (min or hr)
\tau_a  time constant of absorption, assuming a first-order absorption process (min or hr)
t_{1/2el}  half-life of elimination, assuming a one-compartment model with first-order elimination (min or hr)
\tau_{el}  time constant of elimination, assuming a one-compartment model with first-order elimination. Sometimes \tau_{el} is referred to as the mean turnover time (min or hr)
\tau  a time constant corresponding with one or another phase in a pharmacokinetic model; a suffix refers to the phase in question (hr of min)
k  a rate constant (hr^{-1} or min^{-1}); k = \frac{1}{\tau}
A  a pre-exponential factor in pharmacokinetic equations; in one-compartment models with oral administration
\[
A = \frac{FD}{V} \frac{\tau_{el}}{\tau_{el} - \tau_a}
\]
and with i.v. administration A = \frac{D}{V}
\( t_0 \) lag-time, representing the time after oral administration during which (practically) no absorption takes place

\( V_{\text{Ce}l} \) total body clearance function (l/hr or ml/min) including linear as well as non-linear elimination pathways

\( k_{\text{Ce}l} \) the total body clearance constant when only linear mechanisms of elimination prevail (l/hr or ml/min)

\( k_{\text{Cm}} \) metabolic clearance constant; that part of \( k_{\text{Ce}l} \) that occurs by one of more metabolic pathways (l/hr or ml/min)

\( k_{\text{Cr}} \) renal clearance constant, that part of \( k_{\text{Ce}l} \) that occurs by renal excretion (l/hr or ml/min)

\( k_{\text{Ca}} \) 'clearance constant' of absorption (l/hr or ml/min)

\( \frac{dQ}{dt} \) rate of disappearance of the drug (e.g. mg/hr)

\( f_i \) blood flow through the intestine (l/hr or ml/min)

\( f_l \) blood flow through the liver (l/hr or ml/min)

\( K_M \) (apparent) Michaelis-Menten constant of an enzymatic mechanism of drug elimination (mg/l) (dissociation constant)

\( K_T \) (apparent) Michaelis-Menten constant for a tubular secretion process, fully analogous to \( K_M \)

\( K_I \) (apparent) Michaelis-Menten constant for an inhibitor of drug elimination

\( Q_m \) metabolic capacity of an enzyme system (e.g. mg/hr), analogous to \( V_{\text{max}} \) when only one enzyme (homogeneously distributed) is involved

\( T_M \) the transport maximum of a tubular secretion mechanism, having the same meaning for the secretory process as \( Q_m \) has for a metabolic pathway

\( f \) the fraction of the total body clearance that occurs via a potentially saturable pathway

\( AUC \) area under the plasma curve, when plasma concentration data are plotted on a linear scale

\( \Delta t \) dosage interval, when a drug is administered repeatedly (hr or min)

\( \text{pK}_a \) \(-\log K_a\), where \( K_a \) is the acid dissociation constant

\( \text{TPC} \) true partition coefficient; the partition coefficient of the unionized species between an organic solvent and aqueous medium

\( \text{APC} \) apparent partition coefficient (pH-dependent)
CHAPTER 1 GENERAL INTRODUCTION

ANTIPYRETIC AND ANTIINFLAMMATORY ANALGESICS

All substances belonging to this class of drugs are synthetic compounds, derived from the prototypes salicylic acid, phenazone and acetanilide. These prototypes were made in the 19th century, in the search for effective substitutes for quinine. Antipyretic action was the first effect aimed at, but all compounds appeared to have some analgesic effect, which made them useful for the treatment of less severe types of pain. They are not sufficiently active for the treatment of severe pain, so that as a rule they cannot replace narcotic analgesics. One might divide the drugs under consideration in two main groups:

minor analgesics for general use (e.g. paracetamol, phenacetin, glafenine and to some extent acetylsalicylic acid) and

minor analgesics with pronounced antiinflammatory effect (but very low analgesic effect when no inflammatory condition prevails, e.g. most salicylates, arylacetic acid derivatives).

Some of the general analgesics are available without prescription and it needs not to be said that these should meet strong requirements concerning their safety. Apart from the nature of the active substance also the pharmaceutical formulation may be of extreme importance in the respect (see for instance acetylsalicylic acid, chapter 16).

The newer developments, during the last two decades, are especially in the field of the drugs with more specific antiinflammatory action. A lot of arylacetic acid derivatives have been synthesized and tested. Some of these are discussed in chapters 5—9. In another context we reviewed the practical aspects of therapy with antipyretic and antiinflammatory analgesics as well as some current hypotheses concerning their mechanisms of action (van Ginneken, 1975; van Ginneken and van Rossum, 1975). The main mechanisms by which these drugs are supposed to exert their pharmacological effect are briefly indicated in the following paragraph.

ANALGESIC, ANTIINFLAMMATORY AND ANTI PYRETIC EFFECT

Contrary to the narcotic analgesics, which have an effect directly on the central nervous system thereby altering the appreciation and the evaluation of the pain stimulus, the non-narcotic analgesics appear to have a
Peripheral site of action (Lim et al., 1964). Apparently they are competitive antagonists of bradykinin (and possibly other plasma-kinins) which is a potent stimulant of pain chemoreceptors. Bradykinin seems to be responsible for the pain stimulus experienced in several conditions (also in the acute inflammatory reaction) but has a variety of other effects as well and appears to be a major mediator of acute inflammation (Rocha e Silva and Leme, 1972; Rocha e Silva, 1973; Rocha e Silva, 1974; Maling et al., 1974). The apparent relationship between the analgesic and anti-inflammatory effect became still more significant by the discovery that several prostaglandins, known as mediators of inflammatory reactions, had a sensitizing effect on pain receptors, even in concentrations at which these prostaglandins have no pain producing activity themselves (Ferreira, 1972). The minor analgesics are inhibitors of the prostaglandin synthesis and it is especially this effect that seems to be essential for the production of analgesia (Ferreira et al., 1973; Moncada et al., 1975). Several other aspects of inflammatory disorders are mediated or potentiated by prostaglandins as well and the inhibition of prostaglandin synthetase by non-steroid antiinflammatory drugs is very well documented and correlates reasonably with the antiinflammatory potency of the drugs (Ferreira et al., 1971; Vane, 1971; Flower et al., 1972; Vane, 1972; Vane, 1973; Greaves and McDonald-Gibson, 1973; Williams and Morley, 1973; Moncada et al., 1973; Flower, 1974; Flower and Vane, 1974; Newcombe et al., 1974; Deby et al., 1975).

Evidence has been reported that also the antipyretic activity of the drugs, which probably occurs at a central site of action, is explained by inhibition of the prostaglandin synthesis (Flower and Vane, 1972). Several other mechanisms have been proposed as determinants for antiinflammatory activity (see for instance a short review by Paulus and Whitehouse, 1973). Antiinflammatory drugs have been reported to stabilize the membrane of lysosomes, thereby preventing or diminishing the release of lysosomal enzymes from several types of leucocytes, which are involved in inflammation. Although this matter has been extensively studied during the last years, it seems that no unambiguous statement can be made up to now, in view of many conflicting data (Paulus and Whitehouse, 1973; de Duve et al., 1974). However, since several lysosomal enzymes are inhibited also directly by antiinflammatory agents, it seems likely that a decrease of the destructive activity of these enzymes is involved in the mechanism of action (e.g. Ignarro, 1972; Saeed and Warren, 1973; Douwes, 1974; Wojtecka-Lukasik and Dancewitz, 1974; Ringrose et al., 1975; Nakanishi and Goto, 1975). Finally, it should be mentioned that inflammation may
be regarded as an immunologically related reaction and that all known mediators can be incorporated in a unifying concept for inflammation on basis of immunological mechanisms (Willoughby and Di Rosa, 1971, Zvaifler, 1973, Vogt, 1974, Dannenberg, 1975).

As a matter of fact several immunological events appear to be influenced directly by antinflammatory drugs (see references cited above and Brown and Mackey, 1968, Di Rosa et al., 1972, Opelz and Terasaki, 1973, Aisenberg, 1974, Whaley et al., 1975, Crout et al., 1975).

**BASIC STEPS IN DRUG ACTION**

In general drugs may exert their pharmacological action in two different ways. First of all some drugs have an effect by virtue of their overall physicochemical characteristics (e.g. osmotic diuretics, antacids). Usually these are only effective in rather high concentration, since they need to bring about changes in the organism by just being present. The situation is totally different with the large majority of drugs, that give a specific effect originating from the interaction between the drug and some part of the organism for which it has a special affinity. The receptive substance (receptor) can only seldom be adequately defined but has been recognized long ago and provided a useful working hypothesis for molecular pharmacology (see e.g. Anens, 1964). Drug-receptor interactions can fruitfully be described by the law of mass action Many pharmacologists have directed their attention to the processes occurring between the administration of a drug and the observation of its pharmacological effect Most of the experimental work has been done on isolated organ preparations of various origin. The reason for this restriction is obvious in view of the long, largely obscure way from administration of a drug to an intact organism to the ultimately observed effect After drug administration one first has to consider the processes of absorption, distribution and elimination of the drug and the factors that may modify these processes Part of the drug reaches the biophase or receptor compartment where actual interaction between drug and receptor takes place. Only a small fraction of the administered dose will participate in this interaction, while the rest does not react but merely establishes the concentration gradients needed for the achievement of a high enough concentration in the biophase for the effect aimed at. Clearly a much lower dose would suffice if it were administered in or near to the receptor compartment. The least known steps in drug action are those between drug-receptor interaction and effect the stimulus-effect relationship. Real progress in the analysis of these steps can only be made
after integration of pharmacological, physiological and biochemical concepts and data. In an Utopian situation pharmacology would be able to predict for a certain dose of a certain drug not only the nature and the intensity of its effect, but also how fast and how long it would act. Unfortunately we are still far removed from this ideal and therefore the whole area has been divided into the separate study of the basis steps of drug action by the various subdisciplines (pharmacokinetics, biochemical pharmacology, molecular pharmacology, neuropharmacology etc.). Nevertheless some general remarks can be made on basis of our current knowledge. There is a lot of evidence available that the actual drug-receptor interaction takes place in seconds or less (for a discussion on this matter see van Ginneken, 1976). This means that the process of drug-receptor interaction is very fast as compared with the pharmacokinetic processes of absorption, distribution and elimination, which usually have time scales of hours or at least minutes. Furthermore pharmacokinetics is highly significant in that the concentration of the drug in the biophase is somehow related to the plasma concentration, even though the relationship may be very complicated, especially by factors such as specific retention of drug in target tissue, pH differences between plasma and biophase, protein binding etc. In any case the drug-receptor interaction is expected to be a reflection of the pharmacokinetics of the drug rather than determined primarily by its reaction rate constants. There may be some exceptions, for instance some steroid hormones with very low dissociation rate constants of the drug-receptor complex, leading to specific retention of the active complex. However, when the plasma concentration can be followed long enough, also such a retention phenomenon will be reflected in the plasma curve as a final phase with a long halflife. On basis of these considerations one might expect the time course of the pharmacological effect of a drug to be conformable to that of its plasma level. Whereas the plasma concentration usually is a logarithmic function of time, the effect might be rather a linear function of time, since typical dose-response curves show a logarithmic dependence of effect on concentration over substantial dose ranges (see e.g. van Rossum and van Koppen, 1969). It should be noted that this simple relationship can only be found as long as the effectuation of the drug-receptor complex is fast with respect to the pharmacokinetic events, and as long as the effect is not irreversible or only slowly reversible.
THE PRESENT INVESTIGATIONS

In recent years a lot of investigations have been devoted to the mechanism of action of antiinflammatory analgesics (as indicated above). Very little is known, however, concerning the time course of the effects. As a matter of fact the analgesic activity is often difficult to evaluate quantitatively since relief of pain by these rather weak drugs is determined to a large degree by subjective, individual factors which mask more objective parameters. Nevertheless it seems that for several of the drugs a simple relationship between analgesia produced and plasma levels exists. Examples of this will be discussed in some of the following chapters. This implies that pharmacokinetic data are valuable for establishing dosage regimens which may provide effective plasma levels for periods as long as desired. In acute situations relief of pain should be obtained as soon as possible. Therefore also the pharmaceutical formulation of an analgesic can be very important since it determines the rate of absorption of the specific drug. The relevance of pharmacokinetic studies with regard to those aspects of analgesic therapy is obvious. As far as antiinflammatory action is concerned matters may be more complicated in view of the multiplicity of mechanisms involved. Competitive interactions may be supposed to fade away when the drug is disappearing, but more complex interactions are prevailing in antiinflammatory action. For instance, inhibition of a normally occurring process, like the prostaglandin-synthesis, may have a prolonged effect depending upon the turnover-time of the system. Further there may be a complex relationship between the concentration of drug in plasma and at the site of action. Part of the antiinflammatory activity will be located primarily in leucocytes (stabilization of lysosomal membranes, enzyme inhibition, inhibition of lymphocyte transformation). Therefore the concentration of the drugs in the leucocytes will be extremely important and this may be much higher and much more slowly decreasing than the concentration in plasma. Such a phenomenon has been described for colchicine (Wallace and Ertel, 1970) and there is much evidence that several drugs with a carboxylic acid function are selectively taken up by leucocytes (Loh and Wilson, 1970, 1971, 1975). At the moment we are planning investigations into the possibility of uptake mechanisms in leucocytes with high affinity for non-steroid antiinflammatory acids. When a compartment with a prolonged retention of the drugs under consideration exists indeed, this implies that the time course of the effect may be different from the time course of the plasma curve. In principle, such a compartment will be reflected in the plasma curve, but it is doubtful whether it can be detected
in view of the relatively small amounts of drug involved. As a rule therapy with antiinflammatories is guided by clinical symptoms. Several drugs that will be described in the following chapters exhibit highly fluctuating plasma levels in usual dosage regimens. It is a question if these regimens are optimal. One possibility is that the levels at the various sites of action are more constant by retention phenomena as discussed above. Another possibility is that the effect as such reaches a steady level (despite the variable plasma concentration) by long turnover-times of the systems that are influenced. In any case detailed study of the profile of the plasma curve is a first step to the interpretation and evaluation of dosage regimens. With the aid of this information it is possible to adapt regimens for obtaining higher or lower blood levels and to influence the rate at which such levels are approached. Furthermore the pharmacokinetic parameters can contribute to the establishment of dosage regimens that provide blood levels, that are at any time lower than the toxic and higher than the subtherapeutic levels. This is extremely important for instance for salicylates, where the therapeutic concentration is close to the toxic range. Understanding of the mechanisms of the clearance process makes it possible to find out effective measures for treatment of intoxication and also to adjust dosage regimens in case of liver or kidney disturbances (if necessary). The investigations described in this thesis were planned on basis of these general considerations. They have been undertaken from a fundamental pharmacokinetic point of view, but whenever it is relevant, attention is paid to the practical consequences of the findings.

The main point of the present thesis is the interpretation of the pharmacokinetic results in terms of the basic processes that determine the kinetic behaviour of drugs in the body. Special attention is paid to the deviations from normal pharmacokinetic models, such as occur for instance with salicylate.

REFERENCES


The pharmacokinetic analyses described in this thesis are based on measurement of the concentrations of the various drugs in plasma and urine of human volunteers. In this chapter the general procedures are outlined. Details on the way of administration of the drugs and the subjects involved are given in each chapter separately. The subjects were unrestricted as to movement or position. Smoking and water consumption were permitted during the whole course of the trials.

MATERIALS

Ibuprofen, ibufenac as well as 4-n-butylphenylacetic acid were kindly supplied by Boots Pure Drug Company, Nottingham, England; alclofenac by Continental Pharma, Brussels, Belgium; flufenamic acid and mefenamic acid by Parke-Davis, Bornem, Belgium; 4-isopropylaminophenazone by Byk Nederland, Zwanenburg, The Netherlands and 4-methylaminophenazone by Hoechst, Frankfurt, GFR.

Phenazone, 4-isopropylphenazone, 4-dimethylaminophenazone, paracetamol, phenacetin and sodium salicylate were obtained from OPG, Utrecht, The Netherlands.

All materials used for packing of gas chromatographic columns were obtained from Applied Science Lab., State College, Pa., USA.

The silylating reagent BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) was obtained from Pierce, Rockford, Illinois, USA.

Two different types of β-glucuronidase were used (Suc d’Helix Pomatia, Industrie Biologique de France, Gennevilliers, France; and bovine liver glucuronidase, Sigma, St. Louis, Missouri, USA).

All other reagents and solvents were of analytical-reagent grade (Merck, Darmstadt, GFR or Baker, Deventer, The Netherlands).

SAMPLING PROCEDURE

At regular times after drug administration blood samples are taken from a fore arm vein (about 5 ml). The blood samples were centrifuged, plasma was stored in a deep freezer (−20°C) until assay and the erythrocytes were
mostly discarded. In some experiments also the erythrocytes were analysed for their drug content, after they had been homogenized by ultrasonification. For a period of at least 30 hrs after drug intake samples were collected of every aliquot of urine voided. For that purpose the urine was voided in a calibrated cylinder (500 ml), the time and the total volume of the urine was registered, samples were poured into 50 ml bottles, and the rest was discarded. The accuracy of the volume measurement of course is dependent upon the total volume, but the relative error usually will be less than 5%. The pH of the urine was measured as soon as possible and the samples were stored in a refrigerator, when they could be analysed within a few days and otherwise in a deep freezer.

GAS CHROMATOGRAPHIC ANALYSIS

Extraction procedure
For the gas chromatographic methods employed it is necessary to extract the drugs from the biological sample with the aid of a suitable organic solvent. In case of GC analysis always an internal standard (10—20 μg) was added before extraction. When the drug to be extracted was an acid the sample was acidified with 1—3 ml of 3N HC1, when it was a base the sample was alkalinized with 1 ml 1N or 0,2 ml 5N NaOH. The general procedure was as follows: To the plasma (1 ml) or urine (0.2—5 ml, depending upon the concentration of the drug) in a 40 ml conical tube were added: 10—20 μg of an internal standard (if possible in aqueous solution, otherwise in a small volume of ethanol) and the extraction solvent. Then the aqueous layer was acidified or alkalinized or buffered. After mechanical shaking for 20 minutes and centrifugation (if necessary) the organic solvent was transferred to another conical tube and evaporated to dryness with a gentle stream of dry filtered air. When no formation of derivatives was necessary the residu was dissolved in 25—100 μl of absolute ethanol, about 5 μl of which was injected into the gas chromatograph. When the substance had to be derivatized for gas chromatography the residu was dissolved in freshly distilled ether and carefully transferred into a reacti-vial (Pierce).

Formation of derivatives
After slow evaporation of the ether from the reacti-vial, 20—25 μl BSTFA was added and the contents were thoroughly mixed on a whirlmixer. Trimethylsilylation appeared to be practically instantaneous and in any case complete within 1 hr. The silylated products remained stable for at
least two days, when stored in a closed reacti-vial. In practice the deriv­atives (3—5 μl) were injected into the gas chromatograph after standing at room temperature for about 3 hrs. In two cases other derivatives than trimethylsilylated were made, viz. paracetamol and mefenamic acid. Paracetamol was acetylated with acetic anhydride according to the procedure described in detail by Prescott (1971). Mefenamic acid was mostly silylated but in a few cases we prepared the methylester with the aid of trimethylanilinium hydroxide. Trimethylanilinium hydroxide was prepared according to the method of Brochmann-Hanssen and Oke (1969) and was used as a solution (0.1 M) in methanol. The methylation with trimethylanilinium hydroxide takes place after injection in the gas chromatograph in the flash-heater so there is no need for prior incubation. In practice about 1 μl of the methylating solution was taken up in a micro-syringe together with 5 μl of the solution obtained by adding 20—50 μl methanol to the residu of the plasma or urine extraction. This mixture was directly injected into the gas chromatograph.

Gas chromatography
A Hewlett-Packard, Model 402 gas chromatograph equipped with flame ionization detector was used. The column (glass) with a length of 1.80 m and an internal diameter of 3 mm was packed with 3% OV—17 or 3.8% UCW—98 on Gaschrom-Q 60—80 or 80—100 mesh. The gas chromatographic conditions were: nitrogen (carrier gas) flow 20 ml/min, hydrogen flow 30 ml/min and air flow 150 ml/min. These values are indicative; for every specific analysis they were optimalized. The temperature of the oven was adjusted to obtain reasonably short retention times of the compounds eluting from the column, without losing sufficient separation of the peaks. The temperature of the oven was adjusted to obtain reasonably short retention times of the compounds eluting from the column, without losing sufficient separation of the peaks. The temperatures of the flash-heater and of the detector were taken about 50°C and 60°C respectively above the temperature of the oven. The specificity of the analysis was checked by comparison with a lot of blanc urine or plasma samples. Only when in the concentration range studied no interference with peaks originating from endogenous compounds was observed, the procedure was considered adequate. Calibration graphs were constructed by adding variable, known amounts of the drug under investiga­tion together with 10—20 μg of the appropriate internal standard to blanc plasma or blanc urine, treating these samples as described above and plotting the peak area ratio of the drug and its internal standard in the result-
TABLE 2.1

Extraction and gas chromatographic conditions employed in the investigations

<table>
<thead>
<tr>
<th>Substance</th>
<th>Extraction</th>
<th>Deriv.</th>
<th>GC</th>
<th>Internal standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>alclofenac</td>
<td>A, toluene</td>
<td>sil.</td>
<td>UCW 98, 170°C</td>
<td>3,4-dimethoxybenzoic acid</td>
</tr>
<tr>
<td>benzoic acid</td>
<td>A*, ether</td>
<td>sil.</td>
<td>OV 17, 100°C</td>
<td>4-methylbenzoic acid</td>
</tr>
<tr>
<td>ibufenac</td>
<td>A, hexane</td>
<td>sil.</td>
<td>OV 17, 160°C</td>
<td>4-n-butylphenylacetic acid</td>
</tr>
<tr>
<td>ibuprofen</td>
<td>A, hexane</td>
<td>sil.</td>
<td>OV 17, 160°C</td>
<td>4-n-butylphenylacetic acid</td>
</tr>
<tr>
<td>mefenamic acid</td>
<td>A, CHCl₃</td>
<td>sil.</td>
<td>OV 17, 200°C</td>
<td>4-(N-benzylamino)-benzoic acid</td>
</tr>
<tr>
<td>mefenamic acid</td>
<td>A, CHCl₃</td>
<td>me.</td>
<td>SE 30, 220°C</td>
<td>4-(N-benzylamino)-benzoic acid</td>
</tr>
<tr>
<td>phenazone</td>
<td>B, CHCl₃</td>
<td>–</td>
<td>OV 17, 180°C</td>
<td>phenacetin</td>
</tr>
<tr>
<td>4-isopropylphenazone</td>
<td>B, CHCl₃</td>
<td>–</td>
<td>OV 17, 180°C</td>
<td>pheniramine</td>
</tr>
<tr>
<td>4-aminophenazone</td>
<td>B, CHCl₃</td>
<td>–</td>
<td>OV 17, 180°C</td>
<td>4-dimethylaminophenazone</td>
</tr>
<tr>
<td>4-methylaminophenazone</td>
<td>B, CHCl₃</td>
<td>–</td>
<td>OV 17, 180°C</td>
<td>phenacetin</td>
</tr>
<tr>
<td>4-dimethylaminophenazone</td>
<td>B, CHCl₃</td>
<td>–</td>
<td>OV 17, 180°C</td>
<td>phenacetin</td>
</tr>
<tr>
<td>4-isoproplaminophenazone</td>
<td>B, CHCl₃</td>
<td>–</td>
<td>OV 17, 180°C</td>
<td>4-dimethylaminophenazone</td>
</tr>
<tr>
<td>paracetamol</td>
<td>Bu8*, CHCl₃</td>
<td>ac.</td>
<td>OV 17, 180°C</td>
<td>phenacetin</td>
</tr>
<tr>
<td>phenacetin</td>
<td>Bu8, ethylacetate</td>
<td>–</td>
<td>OV 17, 180°C</td>
<td>phenazone</td>
</tr>
</tbody>
</table>

1. A = acidified, B = alkalinized, Bu8 = buffered (pH 8), an asterisk indicates that the aqueous phase was saturated with NaCl in order to enhance extraction efficiency.
2. Derivatives trimethylsilyl (sil.), methyl (me) or acetyl (ac).
3. Gas chromatographic column (3% OV 17 or 3 8% UCW 98 or 2% SE 30 on Gaschrom. Q). Also the temperature of the oven is indicated.
ing chromatogram against the amount of drug added. In most cases the ratio of the peak heights could be used instead of the ratio of the peak areas, since both the relative retention times and the ratio of the peak heights of drug and internal standard appeared to be constant over a wide range of chromatographic conditions. Therefore as a rule it was sufficient to measure peak height ratios instead of the more elaborate surface area determination.

Table 2.1 shows the relevant details concerning preparation of the samples and gas chromatography of the drugs studied.

DISCUSSION

Extraction
Several of the drugs studied are known to be strongly bound to plasma proteins, especially the arylacetic acid derivatives. Therefore, we had to determine whether the method described measures total plasma concentration, including the fraction that is protein bound or only free plasma concentration. Although the latter possibility could be excluded a priori, a possible influence of the presence of proteins in the extraction mixture was investigated by parallel extractions from purely aqueous medium, with the standard added before and after protein denaturation. No interference by plasma proteins was observed in the methods employed. The extraction from plasma was practically the same as from aqueous solution. As expected on basis of the high distribution coefficients (see chapter 3) extraction was essentially complete, the recovery being 95% or more. The same efficiency was found for urine extraction. The internal standards, that were used, were selected on basis of similarity of physicochemical character to the drug under investigation and on basis of their relative retention time in the gas chromatograph with respect to the drug. A primary requirement was of course that it did not coincide with endogenous compounds in the gas chromatogram; further a retention time not too far from that of the drug was required (in practice the difference of the retention times did not exceed a factor 2). Especially in case of acidic compounds that were derivatized for gas chromatography a lot of peaks originating from plasma or urine were observed. Care was taken to avoid interference. Sometimes this required long waiting periods to have all endogenous compounds eluted from the column. Alkaline extracts were much ‘cleaner’ in this respect. When derivatives had to be made for gas chromatography, we judged it essential that the standard was suited to the same derivatization procedure, in order to be a real internal standard. The
use of such internal standards has the enormous advantage that a lot of quantitative pipetting is made superfluous and that calibration is very easy.

**Formation of derivatives**

Although some special columns have been described in literature for the direct gas chromatography of carboxylic acids we were unable to obtain quantitative results with the drugs of our study on such columns. Very good results were obtained by silylation and subsequent gas chromatography. Several very efficient silylating reagents are available.

In our study we used only BSTFA, since this combines powerful donor capacity with high volatility. Also its reaction products are very volatile, so that even at the lowest temperature at which the gas chromatograph was operated (100°C) all waste products eluted from the column together with unreacted BSTFA. Further BSTFA (like several other trimethylsilyl-donors) is an excellent solvent for a variety of organic compounds, especially after silylation. In our studies it appeared unnecessary to use any other solvent. With the exception of paracetamol (see below) no catalyst was used for silylation. The trimethylsilyl derivatives were formed practically instantaneously and were stable for several days, provided that the special reacti-vials (Pierce) were used. As a matter of fact in our experience the use of such vials was essential for the whole procedure since both BSTFA and the silylated compounds are sensitive to moisture. Special measures such as working under nitrogen were not taken, since this appeared to be unnecessary in view of the small volume of the reacti-vials and the large excess of silylating reagent used. Due care was taken to avoid contamination of the BSTFA, the quality of which was checked at regular times with the aid of standard solutions of drug and internal standard.

The acetylation of paracetamol was performed with acetic anhydride (30 μl) and pyridine (15 μl) (Prescott, 1971). The mixture was kept at 45°C for 20 min after which the reaction was virtually complete. This procedure, however, yielded results that suffered sometimes from bad reproducibility. Relative errors as high as 30% were sometimes met in the determinations. Therefore, several parallel measurements were done to obtain a reliable estimate of the error involved in the individual concentration values. These errors were taken into account in the subsequent computerfitting procedures. In our present investigations we obtain much better results with a silylation procedure modified after Prescott (1970) and Thomas and Coldwell (1972). This procedure, consisting of silylation with BSTFA and pyridine (as a catalyst) at 60°C for about 1 hr, yields very reproducible and accurate results.
Mefenamic acid in some experiments was present only in very low concentrations. In these cases it appeared impossible to obtain reliable data with the use of the normal silylation procedure and the flame ionization detector. Since a nitrogen detector made a more sensitive analysis method possible, we adapted the procedure for that purpose. Silylating reagents are not useful for working with a nitrogen detector because of the high risk of contamination of the detector and therefore we used methyl derivatives in those cases. Esterification of the carboxyl group was easily performed with the aid of trimethylanilinium hydroxide according to Brochmann-Hanssen and Oke (1969).

**Calibration graphs**

In all cases linear calibration graphs were obtained for amounts of drug up to 25 µg at least; for the acids up to 50 µg. It need not be said that in any case the magnitude of the sample was adapted to the concentration range over which the calibration graph was constructed. Nearly always plasma samples of 1 ml could be used. Urine samples sometimes were much smaller (especially in case of alclofenac and 4-aminophenazone) and occasionally larger. The calibration graphs were calculated according to the least squares method. The standard deviation of the concentration measurements was calculated and appeared to be about 2% for the arylacetic acids, with the exception of mefenamic acid (≤ 10%) and for the other compounds, except paracetamol (see above), 5% or less. As a rule the standard deviation increased when very low concentrations (< 2 µg/ml) were measured, which was only seldom done, however. The limit of detection was always below 1 µg per sample.

**Alternative procedures**

In literature two alternative extraction and derivatization procedures for gas chromatographic determination of ibuprofen have been described. One implies extraction by passing acidified plasma through a column of Amberlite XAD-2 resin and subsequent esterification with diazomethane (Mills et al., 1973). The other consists of extraction of acidified samples with benzene and methylation with 1,1'-carbonyldiimidazole and triethylamine in methanol (Kaiser and Van Giessen, 1974). We prefer our method, which is derived from a procedure described by Nash et al. (1971), because of its safety, simplicity and ease of handling.

Roncucci et al. (1971) published a method for gas chromatographic determination of alclofenac and its metabolites. They perform extraction with methyl isobutyl ketone, whereas we prefer toluene, which is more
volatile, so that it can be evaporated more easily. Further they employ a mixture of hexamethyldisilazane and trimethylchlorosilane in dioxane as a silylating reagent. In our experience BSTFA is very satisfactory and generally applicable for silylation of a variety of compounds.

Extraction procedures for pyrazolone derivatives have been described by Brodie and Axelrod (1949, 1950), who also gave colorimetric methods for quantitative determination in biological material. Gas chromatographic analyses of phenazone (e.g. Prescott et al., 1973) and 4-dimethylaminophenazone (e.g. Windorfer et al., 1973; Vesell et al., 1975) have been published. Our extraction and gas chromatographic analysis is similar to these and is generally applicable for a large variety of alkaline drugs.

SPECTROPHOTOFUOROMETRIC ANALYSIS

For two compounds a spectrophotofluorometric analysis was used. In fluorometry it is essential to ascertain the concentration range in which the sample to be analysed is lying, since the fluorescence is not unambiguous. As is well-known, at high concentration of the fluorescent compound the relative fluorescence yield diminishes by a quenching phenomenon, so that a calibration graph has the form of a bell-shaped curve. A certain fluorescence may correspond with a concentration above or below the top of the curve. Therefore, the samples were diluted if necessary to check the validity of the readings. The wave lengths of activation and emission were adjusted before every measurement and standard solutions for calibrating purposes were repeatedly examined during each period of measuring. For all measurements an Aminco Bowman spectrophotofluorometer was used.

Salicylic acid

The fluorometric determination of salicylate has been the subject of several papers (e.g. Saltzman, 1948; Chirigos and Udenfriend, 1959; Potter and Guy, 1964; Harris and Riegelman, 1967; Rowland and Riegelman, 1967; Lever and Powell, 1973). The method we used is similar to the one described by Harris and Riegelman and by Rowland and Riegelman and involves the following steps: plasma or urine (usually 0.5–1 ml) is acidified with 0.5 ml 1N H₂SO₄ after adding 10 ml ether (plasma) or 5 ml carbon tetrachloride (urine), which have been saturated with water. The mixture is mechanically shaken for 20 min and then centrifuged. 1 ml of the ether layer (or 0.5 ml of the carbon tetrachloride layer) is reextracted with 5 ml phosphate buffer of pH 7.1, by shaking for 20 min. After centrifugation the ether layer is aspirated and residual ether is removed by
blowing dry filtered air through the buffer solution. In case of extraction with carbontetrachloride the fluorescence of the buffer solution can be read directly after centrifugation (activation wave-length 308 nm, emission wave-length 412 nm). The procedure yields an average recovery of 97 ± 3%. Linear calibration curves can be obtained over a concentration range up to about 100 mg/l plasma or urine and even over much wider ranges by appropriate dilution of the samples. The method is very sensitive, but due to the presence of some fluorescent material in blanc samples accurate readings cannot be done at concentrations below 0.5 mg/l plasma or urine.

**Flufenamic acid**

Our efforts to obtain a reliable gas chromatographic procedure for the assay of flufenamic acid in plasma and urine were unsuccessful because of interfering peaks from blanc plasma. Therefore, we used the fluorometric method of Hattori et al. (1970) with some minor modifications. This method is based on the finding that flufenamic acid gives a strongly fluorescent complex with aluminium chloride in ethanol. Briefly the following steps are involved. Extraction of flufenamic acid from the acidified sample is performed with 10 ml ethylacetate. After mechanical shaking for 20 min the mixture is centrifuged and 2 ml of the ethylacetate-layer is slowly evaporated at 40°C by a gentle stream of dry, filtered air. The residu is reconstituted in 4 ml of absolute ethanol and then 0.1 ml of a solution of 0.5% aluminium chloride in absolute ethanol is added. The fluorescence of the resulting solution at a wave-length of 450 nm is read (activation wave-length 360 nm). The recovery of flufenamic acid in this procedure is essentially complete. The standard deviation of the determinations is 1—5% (increasing with decreasing flufenamic acid concentration). Calibration curves are linear up to at least 10 μg of flufenamic acid added per ml plasma. The sensitivity is high, in alcoholic solution a concentration of 0.01 mg/l can easily be measured. The measurement of flufenamic acid in biological samples, however, is limited by the presence of blanc 'flufenamic acid levels' of about 0.1 mg/l.

**OTHER GENERAL METHODS**

All other methods are given in the text whenever it is relevant, except for the following:

  - Glucuronidase treatment of urine samples.

  In several studies we determined not only the free drug excreted in urine, but also the amount excreted as glucuronide. For this purpose
5000—10,000 units of β-glucuronidase were added per ml of adequately buffered urine (depending upon the pH optimum of the glucuronidase employed). In all cases splitting of the glucuronides was virtually complete after standing at room temperature for about 15 hrs (overnight). The samples were analysed further as described above. By comparing the drug content after glucuronidase treatment with that measured directly, the amount of drug excreted as glucuronide was determined.

**ANALYSIS OF PLASMA CURVES.**

The plasma concentration data were fitted* by computer according to an open one-compartment model with oral administration. In this way optimal estimates for the various pharmacokinetic constants were obtained. Usually visual inspection of the fitted curve as compared to the experimental data convinced us of a very good adaptation to the model which assumes first-order absorption as well as first-order elimination. In other words the simple one-compartment model with first-order rates appeared to be highly acceptable for description of the experimental situation. However, in some instances, the computer program was not able to fit the data in an acceptable way to the linear one-compartment model. In these cases we suspect non-linear absorption to occur. Obviously then the curves cannot be described by a sum of two simple exponential terms. In such cases the computer, being unaware of the model mistakes, can make enormous errors also in the elimination phase, although that always appears to be first order, except of course in those cases where a better approximation (capacity-limited kinetics as for salicylic acid and 4-amino-phenazone) is available. However, when absorption was not first order while elimination was, the time constant for elimination was determined by manual linear regression of the data from the logarithmic phase. The points in the absorption phase cannot easily be adapted to another model, since a general mathematical description of processes that cause deviations from first-order absorption is in most cases impossible. The main processes responsible are:

1. rate limiting dissolution of the drug in the gastrointestinal tract. This can also apply to drugs given in solution since the acidic drugs we deal with can be expected to precipitate in the stomach contents.
2. disturbances by the presence of food etc. in the gastrointestinal system.

Obviously then the access of the drug to the absorbing mucosa can be

* Non-linear regression program FARMFIT, in use at the Computer Centre of the University of Nijmegen.
3. rate limiting processes in the transport mechanisms through the absorbing membranes.

4. reversible absorption, which means that the membrane passage can go in two directions, so that pseudo steady-states can be achieved in the absorption phase.

5. another point of interest is the occurrence of a so-called lagtime. This means that there may be a certain period after administration of the drug, during which no absorption takes place at all or during which the absorption is at least very much slower than first order. It is very common that before a process appears to follow first-order kinetics an induction period takes place during which possible pre-requirements can be fulfilled. For instance, in the case of gastrointestinal absorption one can imagine that first a concentration gradient towards the vascular system should be established before the rate becomes first order.

In practice it would be difficult to discriminate between the various possibilities, especially since usually only a few data points are available in the absorption phase. Therefore, we decided not to try to characterize these deviations further.

Only as far as the lagtime is concerned our standard mathematical analysis by the computer program Farmfit offers the opportunity to decide in an objective manner whether we get a significant improvement by allowing a lagtime to occur or not. In practice any curve is analysed by Farmfit in two ways: one with and one without a lagtime. When by introducing the lagtime a significant decrease in the chi-square value is achieved we assume that the parameters obtained this way are the best estimate, if there is no or a too small decrease in the chi-square value we can safely neglect the possibility of a lagtime. In the tables of parameters we usually did not list lagtimes since there is no way in which they can be interpreted in a quantitative manner. The general mathematical description of the plasma concentration $C$ in a single compartment system with oral administration and first-order kinetics is given by the following equation:

$$C = A \left[ e^{-\frac{(t-t_0)}{\tau_{el}}} - e^{-\frac{(t-t_0)}{\tau_a}} \right]$$

(cf chapter 4, eq. 4.16)

where

$$A = \frac{FD}{V} \frac{\tau_{el}}{\tau_{el} - \tau_a}$$
The computer program Fannfit now gives the best fitting values for $\tau_{el}$, $\tau_a$, $t_0$ (if a lagtime is allowed to occur) and $A$, together with the errors in these parameters. When no lagtime is regarded it is obvious that the error in the intercept $A$ will be in the same order of magnitude as the error in $\tau_{el}$. When, however, a lagtime is introduced also the uncertainty in this parameter will contribute to the error in $A$ and usually this contribution is much larger because only a few data points are available in the absorption phase and because the absorption process not seldom deviates from first-order kinetics. Furthermore one can imagine that when the absorption phase is allowed to be characterized by two parameters (lagtime and the time constant for absorption $\tau_a$) there appears a strong negative correlation between these two parameters if not enough data are available. This correlation is of course of a purely mathematical nature: physiologically and pharmacokinetically lagtime and absorption time constant are fully independent parameters. However, it can increase the uncertainty in the parameters to a large extent. Moreover, in the computer program the parameters are regarded as normally distributed. This is obviously a wrong approximation. It is easy to see that both for the lagtime and for $\tau_a$ the distribution of values to be expected is very asymmetric. In cases where the computer provides errors in the parameters which are unlikely large, we attributed this to the following factors:

1. too few data in the absorption phase
2. asymmetric distribution of expectation-values
3. unallowed extrapolation: in the program the errors are determined by making small changes in the best fitting values and by extrapolation of the deviations obtained from optimal fit.
4. mathematical correlation between estimated parameters: the uncertainty in the parameters of course will increase when they show a strong correlation. This as a matter of fact is a common complication. Quite often the number of data is not very large with respect to the number of parameters to be fitted (for instance 10 : 4) and the curves exhibit too little characteristics (usually only an ascending and a descending limb on basis of two exponentials) to distinguish the parameters clearly enough. As a result of this a mathematical correlation may arise between the various parameters, not only between $\tau_a$ and $A$, as we discussed in some detail, but also in the other combinations of parameters.
REFERENCES

CHAPTER 3 DRUG PARTITIONING

INTRODUCTION

According to common theories in pharmacology the passage of drugs through membranes is restricted to the drug in its unionized form.

Although there is no direct evidence for confirming this hypothesis, it appears a useful assumption in many experimental studies concerning passive diffusion of drugs through biological membranes. Of course, this assumption is unjustified when active, carrier-mediated transport processes are concerned and when the possibility of ion-pair formation has to be regarded. In pharmacokinetics the concept especially applies to the processes of drug absorption and renal drug excretion. Totally in line with the hypothesis are, for instance, the observations that renal excretion of amphetamines is diminished by high urinary pH (Vree, 1973) while that of acids (for instance salicylate) is highly enhanced under these circumstances (see e.g. chapter 16). This pH-partition concept has led to an equation like the one of Henderson — Hasselbalch. This equation, however, can easily lead to misunderstandings, since actually partitioning is determined not only by the concentration of unionized species, so by the pH of the medium and the \( pK_a \) of the drug, but also by the intrinsic lipophilicity of the drug (the lipophilicity of the unionized forms, as reflected in the true partition coefficient TPC, the partition coefficient of the drug in its unionized form). It is well-known that proton transfer is an extremely fast reaction (Eigen, 1963). This implies that ionization equilibria are very stable in the sense that disturbances in the equilibrium concentrations of the reactants are compensated practically instantaneously. Dealing with membrane passage it is obvious that even though the concentration of the unionized molecular species may be very small a substantial amount can penetrate the barrier, provided that its lipophilicity is high. In other words, the unfavourable ionization conditions are compensated by high lipophilicity. pH partitioning as reflected in Henderson — Hasselbalch’s equation of course remains important, since that governs the diffusion gradients in both directions through the membrane. Drawing conclusions from the predictions of the equilibrium concentrations at both sides of the membrane is, however, questionable since obviously in drug absorption and renal excretion one is not dealing with equilibrium situations, but the rate of penetration is the crucial determinant. This rate can be approached by investigating the degree of partitioning of a certain drug between
watery buffers of various pH and an organic solvent. The partition coefficients obtained in such extraction systems indicate how important penetration through the membrane into a constantly moving and refreshing medium will be. This is in fact the most simple system for combining the effects of $pK_a$ and partition coefficient on drug distribution (cf. also Wagner and Sedman, 1973). In this chapter a method will be discussed for determining the true partition coefficient of a drug, when its $pK_a$ is known, of its $pK_a$, when the TPC is known, or even of both $pK_a$ and TPC when very accurate partitioning measurements are performed at many different pH values so that the data can be fitted by computer to a model with the two independent variable parameters.

Our interest is especially in the field of non-steroidal antiinflammatory agents and therefore the substances discussed are all members of this class of drugs. Several of these are aromatic carboxylic acids. Direct measurement of the TPC of these compounds by using a very acidic aqueous phase where ionization is negligible is usually difficult and very inaccurate because of the low solubility of the substances in an acidic medium and because of the rather high TPC. We prefer to use a method which is certainly more laborious, but the results of which are far more significant. In an appendix to this chapter some data are given concerning the partitioning of phenazone derivatives, that are relevant for the discussion in chapter 10.

THEORY

In aqueous environment the following ionization equilibrium is established for acids

$$HA \rightleftharpoons A^- + H^+ \quad (3.1)$$

The degree of ionization is dependent upon the dissociation constant of the acid $K_a$ according to

$$K_a = \frac{[A^-][H^+]}{[HA]} \quad (3.2)$$

After rearrangement:

$$\frac{[HA]}{[HA] + [A^-]} = \frac{[H^+]}{[H^+]+K_a} = \frac{1}{1 + \frac{K_a}{[H^+]}} = \frac{1}{1+10^{pH-pK_a}} \quad (3.3)$$
where $pH = -\log[H^+]$ and $pK_a = -\log K_a$

When we introduce partitioning between the aqueous medium and an equal volume of organic solvent, we can define the extraction ratio in equilibrium situations, $E$, as:

$$E = \frac{C_{org}}{C_{org} + C_w} = \frac{C_{org}/C_w}{1 + C_{org}/C_w} = \frac{APC}{1 + APC} \quad (3.4)$$

where $C_{org}$ = total concentration in the organic phase
$C_w$ = total concentration in the aqueous phase
APC = apparent partition coefficient (pH dependent)

Assuming that only unionized molecules are extracted in the organic phase we obtain under equilibrium conditions:

$$E = \frac{TPC}{1 + 10^{pH - pK_a + TPC}} \quad (3.5)$$

In case of alkaline substances in an analogous way it can be derived that

$$E = \frac{TPC}{1 + 10^{pK_a - pH + TPC}} \quad (3.6)$$

By measuring $E$ at various pH values we can calculate the TPC and/or the $pK_a$ by means of a non-linear curve fitting program (such as Farmfit), which supplies the optimal estimates of the parameters. In our experience it is difficult to fit on basis of the two parameters simultaneously, since then the uncertainty in both of them usually is very high. In general, however, the method should be used for determining TPC for drugs whose $pK_a$ is measured in an independent way. When the $pK_a$ is kept fixed at the known value, the TPC can be determined very accurately. When the TPC is not too large, for instance about 10, which means that $E$ maximally will be about 0.9, then of course the computer program gives quite accurate estimates for both $pK_a$ and TPC. Graphical estimation of the TPC is very easy by measuring the pH at which $E = 0.5$ (50% of the total amount of drug is in the organic phase) since then:

$$pH_{0.5} = pK_a + \log (TPC - 1) \quad (3.7)$$
Obviously this equation is only meaningful when TPC > 1 (otherwise of course E would always be smaller than 0.5). This, however, is practically always the case. When bases are considered instead of acids one would obtain:

\[ \text{pH}_{0.5} = \text{pK}_a - \log (\text{TPC} - 1) \]  

(3.8)

It should be noticed that the TPC which is introduced in this analysis may have only a limited significance from a thermodynamic point of view. As is well-known carboxylic acids can form dimers in organic solvents. In such a case a thorough analysis of the partitioning would require incorporation of the dimerization equilibrium into the equations described here. The true thermodynamic partition coefficient is the ratio of the concentration of unionized monomer in the organic phase over that in the aqueous phase and will be lower than the TPC we estimated, since only part of the substance in the organic phase is present as monomer (depending upon the equilibrium constant of dimerization).

**MATERIALS AND METHODS**

Ibuprofen and ibufenac were obtained from Boots Ltd., Nottingham, England. Alclofenac was obtained from Continental Pharma, Brussels, Belgium. Flufenamic acid and mefenamic acid were obtained from Parke Davis, Bornem, Belgium. All other reagents were Merck p.a. or Baker analyzed reagent grade. Buffer solutions from pH 2 to 9 were prepared according to McIlvaine (citric acid/phosphate) or Teorell and Stenhagen (citrate/phosphate/borate). Stock solutions of drugs were made in the organic solvents in a concentration of 2 mg/ml. Partitioning systems were composed of one milliliter drug solution combined with 9 ml of pure organic solvent (saturated with buffer) and 10 ml of aqueous buffer solution (saturated with organic solvent) of different pH in closed reaction vials. The solutions were thoroughly mixed in a shaking machine for about half an hour and thereafter equilibrated. Equilibrium was reached usually in about two hours, as it appeared from measurements after various equilibration times. After equilibration the pH of the aqueous phase was measured again (in cases that the pH of the buffer solution prepared differed from that after equilibration the latter of course was used for the calculations). 0.2 ml of the organic phase was pipetted, internal standard was added, silylation and GC analysis was performed as described before.
Then the concentration in the organic layer and the extraction ratio could be calculated. As far as the organic solvents are concerned, we used hexane (Merck p.a.) or chloroform. The chloroform used was either chloroform Merck p.a. (indicated CHCl₃ in table 3.1) or freshly distilled chloroform (CHCl₃, dist).

RESULTS AND DISCUSSION

The values for the extraction ratio at the various different pH values were fitted according to equation 3.5, mostly with a fixed pKₐ value but in some cases also with both pKₐ and TPC variable. The results of this analysis are summarized in table 3.1 and some illustrating examples are given in figure 3.1. It should be noted that all partition data at the different pH values were given a weight factor on basis of the standard error in the mean of several determinations. Table 3.1 clearly shows that par-

<table>
<thead>
<tr>
<th>Substance</th>
<th>pKₐ</th>
<th>TPC (± SE)</th>
<th>Organic solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>alclofenac</td>
<td>4.60</td>
<td>77.7 ± 3%</td>
<td>CHCl₃, dist</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.0 ± 10%</td>
<td>CHCl₃, dist</td>
</tr>
<tr>
<td>ibuprofen</td>
<td>4.40</td>
<td>38.1 ± 10%</td>
<td>hexane</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1170 ± 10%</td>
<td>CHCl₃</td>
</tr>
<tr>
<td>ibufenac</td>
<td>4.10</td>
<td>11.1 ± 13%</td>
<td>hexane</td>
</tr>
<tr>
<td></td>
<td></td>
<td>270 ± 12%</td>
<td>CHCl₃</td>
</tr>
<tr>
<td>flufenamic acid</td>
<td>3.90</td>
<td>22330 ± 7%</td>
<td>CHCl₃, dist</td>
</tr>
<tr>
<td>mefenamic acid</td>
<td>4.20</td>
<td>56180 ± 4%</td>
<td>CHCl₃, dist</td>
</tr>
<tr>
<td>flufenamic acid*</td>
<td>3.70</td>
<td>36000 ± 300%</td>
<td>CHCl₃, dist</td>
</tr>
<tr>
<td>mefenamic acid*</td>
<td>4.20</td>
<td>56000 ± 100%</td>
<td>CHCl₃, dist</td>
</tr>
</tbody>
</table>

*fitted on both pKa and TPC; errors are very large by strong mathematical correlation between pKa and TPC.
Partition coefficients are highly dependent upon the organic phase used. This is obvious as far as different solvents are concerned but also the quality and the purity of the solvents is rather critical. This is especially important in case of chloroform to which always about 0.7% of ethanol is added to avoid phosgene formation.

![Diagram showing the distribution of flufenamic acid and mefenamic acid between freshly distilled chloroform and aqueous buffers of different pH. Also the fraction of the acids that is unionized at the various pH values is plotted. Note the shift of the extraction curve with respect to the ionization curve.]

**Figure 3.1**

*Distribution of flufenamic acid and mefenamic acid between freshly distilled chloroform and aqueous buffers of different pH. Also the fraction of the acids that is unionized at the various pH values is plotted. Note the shift of the extraction curve with respect to the ionization curve.*

In Fig. 3.1 also the degree of ionization at the various pH values is given in the form of an ionization curve. Obviously in most instances even in case of practically complete ionization a high extraction ratio is obtained. This indicates a compensation effect of lipophilicity on the unfavourable ionization degree. In an analogous way it might be explained that most of the non-steroidal antiinflammatory acids are for the largest part reabsorbed from tubular urine even when the urinary pH is some units above their pK<sub>a</sub> value. For instance, excretion of salicylic acid (pK<sub>a</sub> = 3) in urine is negligible at urinary pH of 5 although at this pH 99% of the salicylate is in the ionized form. That this very low excretion is due to tubular reabsorption indeed can be deduced from the fact that by increasing urinary pH to 7 a hundredfold enhancement of renal clearance results. An analogous
phenomenon can be expected to occur in case of alkaline drugs that are sufficiently lipophilic in nature. It seems therefore no optimal approach to predict the amount of tubular reabsorption of drugs solely on basis of their $pK_a$ as compared to the pH of the urine. It might be of advantage to use for these purposes the $pH_{0.5}$ as defined in eq. 3.7, since this is an easily interpretable parameter combining both ionization and partition effects. Like any other parameter describing partitioning this $pH_{0.5}$ is dependent on the organic solvent used and only values that have been determined in exactly the same system can be compared.

APPENDIX

PHENAZONE DERIVATIVES

In view of the variability of the estimates of partition coefficients with varying quality of the organic solvent used for extraction, we determined the distribution of the phenazone derivatives used in our study all together under identical circumstances instead of taking data from various sources in literature.

MATERIALS AND METHODS

For the phenazone derivatives see chapter 2. The chloroform used was one and the same lot of chloroform Merck p.a. A phosphate buffer of pH 7.4 was prepared according to Sørensen. Partitioning systems were the same as described before in this chapter. Quantitative analysis was done by injecting the same volume of the organic layer before and after extraction with buffer solution into the gas chromatograph (see chapter 2). These injections were performed repeatedly in order to obtain reliable results. The extraction ratio was calculated and from that the apparent partition coefficient at pH 7.4.

RESULTS AND DISCUSSION

The values obtained, together with their standard deviation are given in table 3.2. All partition experiments were done at least 6 times and each sample was injected 10–12 times. The partition coefficient of 4-isopropylphenazone could not be determined by the method applied since the addition of the phosphate buffer had no significant effect on the concentration in the organic layer. By using systems with volumes of phosphate buffer 2, 4, 6 and 8 times as high as the volume of the organic layer and by
extrapolating the partition ratios obtained to the situation of equal volumes, the partition coefficient could be roughly estimated at 150 (± 30).

TABLE 3.2

Apparent partition coefficient (chloroform-aqueous buffer, pH 7.4) of some phenazine derivatives.

<table>
<thead>
<tr>
<th>Substance</th>
<th>APC(_{7.4}) ± SD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenazine</td>
<td>19.4 ± 5.1</td>
</tr>
<tr>
<td>4-isopropyphenzone</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>4-aminophenazole</td>
<td>12.7 ± 0.8</td>
</tr>
<tr>
<td>4-methylaminophenazole</td>
<td>6.7 ± 1.4</td>
</tr>
<tr>
<td>4-dimethylaminophenazole</td>
<td>13.4 ± 3.1</td>
</tr>
<tr>
<td>4-isopropylaminophenazole</td>
<td>8.8 ± 1.3</td>
</tr>
</tbody>
</table>

REFERENCES


SECTION II
LINEAR PHARMACOKINETICS
INTRODUCTION

The absorption and elimination of drugs usually is supposed to be governed by first-order processes. This means that all rate processes can be described by linear differential equations which as a rule can easily be integrated. Sometimes, however, deviations from this first-order behaviour are encountered and then one is dealing with non-linear pharmacokinetics. For some cases of non-linear pharmacokinetic behaviour a reasonable formal mathematical description is possible. Examples are the so-called capacity limited elimination (when the enzyme system, responsible for elimination by metabolism, can become saturated), active tubular secretion and/or active tubular reabsorption in the kidney and binding of drug to serum proteins (when binding is strong enough to become the rate limiting step in drug elimination or distribution). Some aspects of non-linear pharmacokinetics are discussed in more detail in section III. Here we confine ourselves to a broad outline of linear pharmacokinetics as far as it is relevant for understanding the data and the analysis presented in section II. More specific aspects will be discussed in the following chapters, whenever they are important for understanding and describing the experimental data. Concerning the elementary basis and significance several reviews and textbooks are available (e.g. Dost, 1953; Rescigno and Segre, 1961; van Rossum, 1971; Wagner, 1971; Notari, 1975; Gibaldi and Perrier, 1975). In this chapter some new derivations and theoretical results are included.

CLEARANCE

The most fundamental concept in pharmacokinetics is the clearance, the sum of the processes by which a drug is removed from the body. The parameter to characterize the clearance process can be called the clearance function, $V_{Cel}$. Usually ‘clearance’ refers to removal of the drug from the body compartment in which the drug concentration is followed, so in general one should express this by speaking of plasma clearance (when plasma concentrations are measured) or blood clearance (when concentration in whole blood is measured). Unless explicitly stated otherwise, by clearance we mean the plasma clearance.

The clearance function $V_{Cel}$ may be very complex, but when it can be
properly defined the rate of elimination of a drug is simply related to the plasma concentration at any moment:

\[
\frac{dQ}{dt} = - \hat{V}_{\text{Cel}} \cdot C
\]  

(4.1)

where \(Q\) is the amount of (unchanged) drug in the body compartment, from which elimination occurs, at any time \(t\);
\(C\) is the plasma concentration at any \(t\);
\(\hat{V}_{\text{Cel}}\) has the dimension of \(1/\text{hr}\) or \(\text{ml/min}\).

Since in this chapter we will confine the discussion to linear kinetics we can define the clearance function as a clearance constant \(k_{\text{Cel}}\). So:

\[
\frac{dQ}{dt} = - k_{\text{Cel}} \cdot C
\]  

(4.2)

The clearance constant \(k_{\text{Cel}}\) can in an abstract sense be regarded as the volume that is fully cleared from drug per unit of time.

On basis of this equation we can define the amount of drug eliminated at a certain time after administration, \(Q_{\text{el},t}\) as:

\[
Q_{\text{el},t} = k_{\text{Cel}} \int_{0}^{t} C \, dt
\]  

(4.3)

Ultimately, when \(t\) approaches \(\infty\) or at least is large with respect to the slowest elimination process involved, the whole dose \((D)\) administered will be eliminated; in formula:

\[
D = k_{\text{Cel}} \int_{0}^{\infty} C \, dt
\]  

(4.4)

The integral in the right side of equation 4.4 of course is nothing else than the area under the curve (AUC) of the plasma concentration plotted versus time on a linear scale.

\[
k_{\text{Cel}} = \frac{D}{\text{AUC}}
\]  

(4.5)

This implies that the clearance constant in principle can be calculated after graphical determination of the AUC from \(t=0\) to \(t=\infty\). This may, however, be very difficult especially when the profile of the plasma curve is dependent upon various processes with a highly different rate, which implies
that the decay at low plasma concentrations cannot be predicted accurately and that large mistakes may be made in the estimation. How large these mistakes can be, will become more clear from the following parts of this chapter. A general problem is furthermore that in case of oral administration one cannot be sure that the whole dose has reached the circulation, so that D, which is of course the dose that has come into the body, is not known exactly. The main advantage of the use of eq. 4.5 for calculating the clearance constant is that it is to some degree a model-independent procedure which is valid as long as linear kinetics prevail. Of course the clearance function will not be a constant anymore, but a dose-dependent parameter when non-linear processes are responsible for (part of) the elimination of the drug.

VOLUME OF DISTRIBUTION

When a drug is administered it will always have to come first in the blood before it can be transported to wherever its action is needed. Of course there are some exceptions: desinfectants, antacids and some other gastrointestinal drugs can exert their effect without passing through the bloodstream and such a passage should even be avoided in many cases. Furthermore, there are some drugs like local anaesthetics that can be applied directly to the site of action and sometimes even other drugs are given systemically to insure localization of the drug whose effect is primarily required. There are, however, only a few types of drugs that can be administered so near to the site of action that they need not to be distributed by the blood. For the large majority of drugs administration is always, directly or indirectly, to the bloodstream from where distribution, including transport to the site of action, and elimination occurs. So the fact that in practice only blood is accessible as a real body tissue for regular drug concentration measurements is not a disastrous limitation since the blood as the primary transport system of the body gets in contact with all other tissues and will equilibrate more or less rapidly with all of them. The rate of equilibration between the various tissues with respect to their drug content of course will be determined not only by their degree of perfusion and their volumes but also by the physicochemical characteristics of the drug.

In pharmacokinetics the body is assumed to be composed of one or more compartments. The drug concentration within such a compartment may be inhomogeneously distributed over the various tissue and water components, but a requirement is that within the compartment a concen-
tration equilibrium exists which is established very fast with respect to transfer in and out the compartment (in other words: equilibrium may not be disturbed by interaction with other compartments). As we will see the number of compartments needed in a certain model to describe concentration data adequately is highly dependent on the accuracy and the sensitivity of the assay method. Obviously the central compartment (and often the only one) is that compartment which is constantly in equilibrium with the blood. This compartment has a certain apparent volume which can be defined as that volume over which the amount of drug present in it should be distributed if the concentration all over the compartment would equal the plasma concentration. When more compartments are present each of these will have such an apparent volume.

Sometimes the volume of distribution of a drug can be interpreted as the volume of a real body compartment, for instance plasma, extracellular, water or total body water (about 3 l, about 12 l and 30-40 l respectively). Some drugs can even be used for assessment of the volume of total body water, e.g. phenazone and some of its derivatives. Mostly, however, the distribution volumes encountered are composites of these, what one might call elementary volumes, and often the distribution volume is much larger by accumulation in one or another tissue.

**ONE-COMPARTMENT MODEL**

In many cases pharmacokinetic analysis on basis of only one compartment is sufficient. The rate of elimination of a drug from the body as a single compartment is very simple:

\[ \frac{dQ}{dt} = -k_{Cel} \cdot C \]  \hspace{1cm} (4.2)

This equation can easily be integrated after dividing both sides by the apparent volume \( V \) and after establishing the boundary conditions

\[ \frac{dC}{dt} = -\frac{k_{Cel}}{V} \cdot C \]  \hspace{1cm} (4.6)

The factor \( \frac{k_{Cel}}{V} \) has the dimension of \( hr^{-1} \) (or \( min^{-1} \)) and is a rate constant. This rate constant \( k \) is used extensively in literature as the basic pharmacokinetic parameter (This, however, seems no optimal approach from an educational and conceptual point of view). We often use a time constant:

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\[ \tau_{el} = \frac{1}{k} = \frac{V}{k_{Cel}} \text{ (dimension hr or min)} \] (4.7)

This time constant again has the advantage of being easily interpretable, it is the time in which the whole volume has passed through the eliminating organ(s) once. Furthermore, it is analogous to the half-life \( t_{1/2_{el}} \) which in practice is the most commonly used parameter and which is also a time constant.

**SINGLE COMPARTMENT, INTRAVENOUS ADMINISTRATION**

When the dose \( D \) is administered i.v. the boundary condition for eq. 4.6 is \( (t=0; \ C = \frac{D}{V}) \) and integration yields the well-known formula:

\[ C = \frac{D}{V} e^{-\frac{k_{Cel}}{V} t} = \frac{D}{V} e^{-k \cdot t} = \frac{D}{V} e^{-t/\tau_{el}} = \]

\[ = \frac{D}{V} 2^{-t/t_{1/2_{el}}} \] (4.8)

From this we also see the relation between \( t_{1/2_{el}} \) and \( \tau_{el} \)

\[ e^{-t/\tau_{el}} = 2^{-t/t_{1/2_{el}}} \]

or

\[ t_{1/2_{el}} = \tau_{el} \ln 2 \] (4.9)

**SINGLE COMPARTMENT, ORAL ADMINISTRATION**

When the drug is administered orally (or s.c., i.m., i.p. etc.) eq. 4.2 becomes more complicated, since it must contain also an absorption term. Then a so-called zero’th compartment is introduced (usually the gastrointestinal tract), with a (apparent) volume \( V_0 \), containing an amount \( Q_0 \) with concentration \( C_0 \) (\( =Q_0/V_0 \)). Absorption is supposed to occur via a first-order process governed by the same kind of parameters as the elimination process, but with a suffix ‘a’ attached to the symbols instead of the suffix ‘el’. Such a model can be pictured as:
The model is fully characterized by two differential equations with the appropriate boundary conditions:

\[
\frac{dQ_0}{dt} = -k_{Ca} C_0 \quad \text{[}t=0; Q_0=0\text{]} \quad (4.11)
\]

\[
\frac{dQ_1}{dt} = +k_{Ca} C_0 - k_{Cel} C_1 \quad \text{[}t=0; Q_1=0\text{]} \quad (4.12)
\]

Division by \(V_0\) and \(V_1\) respectively leads to:

\[
\frac{dC_0}{dt} = - \frac{k_{Ca}}{V_0} C_0 = - \frac{C_0}{\tau_a} \quad \text{[}t=0; C_0=\frac{D}{V_0}\text{]} \quad (4.13)
\]

\[
\frac{dC_1}{dt} = \frac{k_{Ca}}{V_1} C_0 - \frac{k_{Cel}}{V_1} C_1 \quad \text{[}t=0; C_1=0\text{]} \quad (4.14)
\]

These simultaneous equations can be integrated very easily. For instance eq. 4.13 can be integrated directly since it is a simple homogeneous first-order differential equation; the result can be substituted in eq. 4.14 which in turn then becomes a first order differential equation (inhomogeneous). In general the solution of a set of simultaneous linear differential equations is easily performed by use of Laplace transformation. Integration of eq. 4.13 and eq. 4.14 yields:

\[
C_1 = \frac{D}{V_1} \frac{\tau_{el}}{\tau_{el} - \tau_a} \left[ e^{-t/\tau_{el}} - e^{-t/\tau_a} \right] \quad (4.15)
\]
Equation 4.15 is still not totally correct and two other parameters have to be introduced in order to make it generally applicable.

1. **The biological availability, F.**

   When a drug is introduced into the gastrointestinal tract it is possible that only part of the dose is absorbed, the rest being excreted unchanged in the faeces. Also it is possible that the drug is absorbed completely, but is metabolized partly at its first passage through the liver before it can reach the general circulation (first-pass effect). For, as is well-known, all substances absorbed from the gastrointestinal tract are collected in the portal vein and go through the liver before being distributed over the body. Still another possibility is that part of the drug is metabolized in the intestine by the microflora before absorption can take place. All these factors can diminish the dose administered effectively and so equation 4.15 will have to be corrected for these phenomena. This usually is accomplished by multiplying the dose $D$ in equation 4.15 with a factor $F$, the biological availability. $F$ or the biological availability can be defined as that fraction of the dose given that reaches the general circulation intact. In case of intravenous administration $F$ will equal unity.

2. **The lag-time, $t_0$**

   Another effect that has to be accounted for is the lag-time ($t_0$). This lag-time can be interpreted as the time after oral administration during which absorption does not yet take place. In a physiological sense its meaning will not always be clearcut. The best approach probably is to regard the lag-time as a purely operational parameter used for fitting absorption data to a first-order model. When, for instance, a solid dosage form is applied one can imagine that before absorption can follow first order kinetics the drug must dissolve and a concentration gradient between gastrointestinal tract and blood must be established. The same can happen when a drug is given in solution but precipitates in the stomach (for instance to be expected with acidic compounds).

   It will take some time (the induction period) before a steady state is achieved. During the induction period some absorption takes place already, but at a very low rate. This implies also that sometimes a measurable plasma concentration can be reached during the lag-time. So in this sense the lag-time is used to correct for deviations from first-order absorption. On the other hand it is conceivable that the presence of food in some cases can retard the diffusion of the drug to the absorbing mucosa in the gastrointestinal tract. Then it will take some time before absorption
can take place anyway.

Furthermore, most drugs will be absorbed primarily from the small intestine, because of the large absorbing surface and not from the stomach, where they come first after ingestion. Although our volunteers usually were fasting overnight before drug administration the passage time through the stomach might be dependent upon the dosage form and on the pH of the stomach contents.

All these factors together are accounted for by the lag-time. We will not do any effort to interpret this lag-time in detail, since we use it as a purely operational parameter which serves to cover irregularities in the absorption process.

Introduction of these two additional factors in eq. 4.15 leads to:

\[ C_1 = \frac{FD}{V_1} \frac{\tau_{el}}{\tau_{el} - \tau_a} \left[ e^{-\frac{(t-t_0)}{\tau_{el}}} - e^{-\frac{(t-t_0)}{\tau_a}} \right] \]  

Two remarks have to be made now:
Firstly, this equation of course is not valid when \( t < t_0 \), then \( C_1 = 0 \).
Secondly, it is obvious that from this equation (so after oral administration) \( V_1 \) (and \( k_{Ce|l} \)) can never be determined absolutely, but only the relative values \( V_1/F \) (and \( k_{Ce|l}/F \)). The absolute values can only be calculated when one can be sure that the whole dose administered has entered the general circulation (as with i.v. administration). The time constants (and the rate constants) of course are independent of the biological availability and can be determined always accurately, provided that enough data points are available.

Equation 4.15 does not apply to the case that \( \tau_a = \tau_{el} \). Then it can easily be derived that:

\[ C_1 = \frac{FD}{V_1} \frac{t}{\tau} e^{-t/\tau} \]  

\( (\tau = \tau_{el} = \tau_a) \)

So instead of a bi-exponential function we get a mixed linear and monoeaxponential function of time. The profile of the plasma curve, however, is quite similar to a bi-exponential one. Fig. 4.1 shows an illustrative example where experimental data are analysed according to both equations and figure 4.2 gives a theoretical example. In practice, when using a computer program for fitting procedures, one should realize that eq. 4.17 might be a better alternative when \( \tau_a \) and \( \tau_{el} \) appear to be nearly equal or when abnormal values for the kinetic parameters are obtained.
Figure 4.1
Regression analysis of experimental data according to equations 4.17 (resulting in $\tau_a = \tau_{el} = 1.48$ hr) and 4.16 (resulting in $\tau_a = 0.6$ hr and $\tau_{el} = 2.2$ hr). Although the parameters differ substantially, the experimental data are reasonably fitted by both curves.

Figure 4.2
Simulated plasma curves for an oral dose of 500 mg of a hypothetical drug ($V = 6$ l, $\tau_{el} = 150$ min) where the time constant of absorption $\tau_a$ increases and ultimately equals $\tau_{el}$. 
MULTICOMPARTMENT MODELS

So far the discussion has been restricted to cases where the one-compartment model may be applied. In fact this implies a rapid distribution over all tissues where the drug can penetrate. This is not seldom the case; especially drugs with a small volume of distribution as caused for instance by strong binding to plasma proteins or by inability to penetrate cells (so that only the extracellular fluid constitutes the distribution volume) will often follow single-compartment kinetics. Also drugs that distribute easily over the total body water often fulfil this criterion. Many drugs exhibit a biphasic plasma concentration curve after i.v. administration and a monophasic curve after oral administration. The reason for this is undoubtedly that after i.v. administration there is a small but measurable period during which distribution from a central compartment (blood and highly perfused tissues) towards another compartment takes place. Provided that this distribution process is more rapid than the absorption process the phenomenon will not show up after oral administration. Sometimes also oral administration data cannot be sufficiently be described by one-compartment models. Then one or even more deeper compartments have to be assumed. The first and central compartment as a rule consists of the blood and highly vascularised tissues (liver, kidney, lung, heart and sometimes brain). This is the compartment in which the concentration measurements are performed and from which elimination is supposed to occur. Another compartment can be regarded as the less perfused tissues (fat for instance), tissues where specific uptake of drug takes place or tissues where drug penetration is hindered by some kind of barrier (brain, placenta). Whether more than one compartment would be discovered or not depends primarily upon the amount of drug involved and the rates of exchange. The influence on the plasma concentration curve will be very clear when a large amount of drug leaves the central compartment to penetrate in another compartment, but will be negligible when only a small amount of drug is concerned. It is also conceivable that when the exchange of drug is fast, compartments might easily be overlooked whereas they can be detected when the rate of exchange is low. When several different compartments show about the same rate of exchange with the central compartment they will not show up as different entities, but they will combine to one. Only rarely more than two compartments will be needed to characterize the kinetic behaviour of drugs in the body. An important factor in this respect obviously is also the accuracy and the sensitivity of the assay method.

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Multicompartment models usually are regarded as a central compartment, from which the elimination processes take place and one or more peripheral compartments which are all in exchange with the central compartment but not with each other. Of course various different types of models are conceivable. For instance, it might be possible that elimination takes place from a peripheral compartment rather than from the central one, and one can think of models where peripheral compartments are connected directly to other peripheral compartments and only indirectly to the central compartment.

MATHEMATICAL ANALYSIS OF MULTICOMPARTMENT MODELS

As long as linear (first-order) kinetics prevail all multicompartment pharmacokinetic models can be described by a set of simultaneous linear differential equations, which can always be solved.

We will outline the general approach for models, in which the drug is administered directly or indirectly to a central compartment 1 which is in exchange with n-1 other compartments, numbered 2 . . . n. These other compartments are not connected among each other and elimination occurs exclusively from the central compartment. The analysis is based on the general approach as described for instance by Rescigno and Segre (1961) and is not restricted to this specific type of pharmacokinetic models, but since this type seems to be the most fruitful instrument in pharmacokinetic practice we will take it as an example. The model under consideration usually is referred to as a mamillary model.
I. INTRAVENOUS ADMINISTRATION

In case of i.v. administration the model is described by the following set of differential equations:

\[
\frac{dQ_i}{dt} = -k_{C1}C_1 - \sum_{i=2}^{n} k_{1i}C_i + \sum_{i=2}^{n} k_{li}C_i \quad \text{[t=0; } Q_i=D]\]

\[
\frac{dQ_2}{dt} = k_{12}C_2 - k_{21}C_2
\]

\[\vdots\]

\[
\frac{dQ_i}{dt} = k_{1i}C_i - k_{li}C_i \quad \text{[t=0; } Q_3=\cdots=Q_i=\cdots=Q_n=0]\]

\[\vdots\]

\[
\frac{dQ_n}{dt} = k_{1n}C_n - k_{n1}C_n \quad \text{(4.18)}
\]

\[Q_i = \text{the amount of drug in the } i\text{th compartment}\]

\[k_{ij} \text{ and } k_{ji} \text{ are parameters in term of clearance constants (with dimension } l/hr \text{ or } ml/min \text{) governing transport in and out the various compartments.}\]

\[C_i = \text{the concentration of drug in the } i\text{th compartment}\]

When we now introduce the volumes of the various compartments \((V_i)\) and apply Laplace transformation we obtain the following set of equations in which \(x_i\) is the Laplace transform of \(C_i\) and \(s\) is the Laplace number.

\[
x_1 = \frac{D}{V_1} - \frac{k_{C1}}{V_1} x_1 - \sum_{i=2}^{n} \frac{k_{1i}}{V_i} x_1 + \sum_{i=2}^{n} \frac{k_{li}}{V_i} x_i
\]

\[
x_2 = \frac{k_{12}}{V_2} x_1 - \frac{k_{21}}{V_2} x_2
\]

\[\vdots\]

\[
x_i = \frac{k_{1i}}{V_i} x_1 - \frac{k_{i1}}{V_i} x_i \quad \text{(4.19)}
\]

\[\vdots\]

\[
x_n = \frac{k_{1n}}{V_n} x_1 - \frac{k_{n1}}{V_n} x_n
\]
Upon rearrangement the following set results:

\[
\begin{align*}
(s + \frac{k_{Cel}}{V_1} + \sum_{i=2}^{n} \frac{k_{i,i}}{V_i}) x_1 - \sum_{i=2}^{n} \frac{k_{i,i}}{V_i} x_i &= = D/V_i \\
- \frac{k_{1,i}}{V_i} x_1 + (s + \frac{k_{1,i}}{V_i}) x_i &= = 0 \\
- \frac{k_{1,n}}{V_n} x_1 + (s + \frac{k_{n,i}}{V_n}) x_n &= = 0
\end{align*}
\] (4.20)

The solution of this set of linear equations is given by the following general equation obtained after application of Cramers rule:

\[
\frac{x_i}{D/V_1} = \frac{\Delta_{1,i}}{\Delta} (-1)^{i+1}
\] (4.21)

where \(\Delta\) is the determinant of the set of equations and \(\Delta_{1,i}\) the determinant which is obtained from \(\Delta\) by deleting the first row and the ith column.

\[
\Delta = \begin{vmatrix}
s + \frac{k_{Cel}}{V_1} + \sum_{i=2}^{n} \frac{k_{i,i}}{V_i} & - \frac{k_{2,i}}{V_2} & \cdots & - \frac{k_{i,i}}{V_i} & \cdots & - \frac{k_{n,i}}{V_n} \\
- \frac{k_{1,2}}{V_2} & s + \frac{k_{2,2}}{V_2} & & & & \\
\vdots & & \ddots & & & \\
- \frac{k_{i,i}}{V_i} & \cdots & s + \frac{k_{i,i}}{V_i} & & & \\
- \frac{k_{i,n}}{V_n} & \cdots & \cdots & s + \frac{k_{n,n}}{V_n}
\end{vmatrix}
\] (4.22)
This determinant can be developed in powers of \( s \) leading to:

\[
\Delta = s^n + H_1 s^{n-1} + H_2 s^{n-2} + \ldots + H_{n-1} s + H_n \quad (4.23)
\]

where \( H_i \) denotes the sum of all minors with rank \( i \) of the determinant obtained from \( \Delta \) by deleting all \( s \) terms, that have the same diagonal elements as that determinant itself. For instance.

\[
H_1 = \frac{\sum V_1}{V_1} + \sum_{i=2}^{n} \frac{k_{11}}{V_1} + \sum_{i=2}^{n} \frac{k_{11}}{V_1}
\]

\[
H_2 = \sum_{i=2}^{n} \left[ \left( \frac{\sum V_1}{V_1} + \sum_{i=2}^{n} \frac{k_{11}}{V_1} \right) \frac{k_{11}}{V_1} \right] + \sum_{i=3}^{n} \frac{k_{21}}{V_2} + \sum_{i=3}^{n} \frac{k_{21}}{V_2} + \sum_{i=4}^{n} \frac{k_{11}}{V_1}
\]

\[
+ \ldots + \sum_{i=3}^{n} \frac{k_{n-1,1}}{V_{n-1}} \frac{k_{n1}}{V_n} - \frac{k_{12} k_{21}}{V_1 V_2}
\quad (4.24)
\]

The determinants \( \Delta_{11} \) are represented by the following general equation.

\[
\Delta_{11} = 
\begin{vmatrix}
-k_{12} & s + \frac{k_{21}}{V_2} \\
& \ddots \\
& & -k_{1,1-1} \\
& & \frac{k_{1,1}}{V_{1-1}} & s + \frac{k_{1-1,1}}{V_{1-1}} \\
& & \frac{k_{1,1+1}}{V_{1+1}} & \ddots \\
& & \frac{k_{1n}}{V_n} & \ddots \\
& & & s + \frac{k_{n1}}{V_n}
\end{vmatrix}
\quad (4.25)
\]
By developing this determinant to the $i$th row it can easily be deduced that

$$\Delta_{11} = (-1)^{i+1} \frac{k_{11}}{V_1} (s + \frac{k_{21}}{V_2}) \ldots \cdot (s + \frac{k_{i-1,1}}{V_{i-1}}) (s + \frac{k_{i+1,1}}{V_{i+1}}) \ldots$$

$$\ldots \cdot (s + \frac{k_{n1}}{V_n})$$

(4.26)

So the general solution for $x_i$ takes the form

$$x_i = \frac{D}{V_1} \frac{k_{11}}{V_1} (s + \frac{k_{21}}{V_2}) \ldots \cdot (s + \frac{k_{i-1,1}}{V_{i-1}}) (s + \frac{k_{i+1,1}}{V_{i+1}}) \ldots \cdot (s + \frac{k_{n1}}{V_n})$$

$$\frac{D}{V_1} \frac{s}{s^2 + H_1 s^{n-1} + H_2 s^{n-2} + \ldots + H_n - s + H_n}$$

(4.27)

It should be noted that $2 < i < n$, so $x_i$ has to be defined separately, but the determinant $\Delta_{1,i+1}$ is a very simple one. It is obviously

$$x_1 = \frac{D}{V_1} \frac{\Delta_{11}}{\Delta} = \frac{D}{V_1} \frac{(s + \frac{k_{21}}{V_2}) \ldots \cdot (s + \frac{k_{i-1,1}}{V_{i-1}}) \ldots \cdot (s + \frac{k_{n1}}{V_n})}{s^2 + H_1 s^{n-1} + H_2 s^{n-2} + \ldots + H_n - s + H_n}$$

(4.28)

One can see that $x_i$ is simply related to $x_1$

$$x_i = \frac{k_{i1}/V_i}{s + \frac{k_{i1}}{V_i}} x_1$$

(4.29)

In order to obtain the solution of the model in terms of $C_i$ we need the antitransform of the functions $x_i$. These can also be given in general equations. The denominator of the equations for all $x_i$ terms is the same. In the mamillar type of models this denominator can always be factorized, so

$$\frac{s}{s^2 + H_1 s^{n-1} + \ldots + H_n - s + H_n} = (s + \frac{1}{\tau_1}) (s + \frac{1}{\tau_2}) \ldots \cdot (s + \frac{1}{\tau_n}) \ldots$$

(4.30)
(The reason for indicating the roots with \( \frac{1}{\tau_i} \) will become obvious from the following).

It is clear from this equation that the \( \tau_i \)'s are functions of all the elementary \( k_i \) and \( V_i \) values in the model, as these are comprised in the coefficients \( H_i \).

As far as the numerator of equation 4.28 is concerned:

for \( x_1 \) this can be written as

\[
\frac{D}{V_1} \left( s^{n-1} + \lambda_{12} s^{n-2} + \ldots + \lambda_{11} s^1 + \ldots + \lambda_{1,n-1} s + \lambda_{1n} \right)
\]

where every \( \lambda_1 \) is a function of all terms \( \frac{k_{i1}}{V_i} \)

and \( \lambda_{11} = \frac{k_{31} k_{21} \ldots k_{n1}}{V_3 V_2 \ldots V_n} \) (4.32)

and for \( x_i \)

\[
\frac{D}{V_1} \frac{k_{i1}}{V_i} \left( s^{n-1} + \lambda_{i2} s^{n-2} + \ldots + \lambda_{ij} s^j + \ldots + \lambda_{i,n-1} s + \lambda_{in} \right)
\]

where \( \lambda_1 \) is a function of the terms \( \frac{k_{21}}{V_2}, \frac{k_{31}}{V_3}, \ldots \) etc. (except \( \frac{k_{i1}}{V_i} \))

and \( \lambda_{1n} = \frac{k_{21} k_{31} \ldots (k_{i-1,1}) (k_{i+1,1}) \ldots k_{n1}}{V_3 V_2 \ldots V_i-1 V_i+1 \ldots V_n} \) (4.34)

So

\[
x_1 = \frac{\frac{D}{V_1} \left( s^{n-1} + \lambda_{12} s^{n-2} + \ldots + \lambda_{11} s^1 + \ldots + \lambda_{1,n-1} s + \lambda_{1n} \right)}{(s + \frac{1}{\tau_1})(s + \frac{1}{\tau_2}) \ldots \ldots \ldots (s + \frac{1}{\tau_i}) \ldots \ldots \ldots (s + \frac{1}{\tau_n})}
\]

(4.35)

\[
x_i = \frac{\frac{D}{V_1} \frac{k_{i1}}{V_i} \left( s^{n-1} + \lambda_{i2} s^{n-2} + \ldots + \lambda_{ij} s^j + \ldots + \lambda_{i,n-1} s + \lambda_{in} \right)}{(s + \frac{1}{\tau_1})(s + \frac{1}{\tau_2}) \ldots \ldots \ldots (s + \frac{1}{\tau_i}) \ldots \ldots \ldots (s + \frac{1}{\tau_n})}
\]

(4.36)
The antittransforms of these equations are given by

\[
C_i = \frac{D}{V_1} \left[ \left( -\frac{1}{\tau_1} \right)^{n-1} + \lambda_{12} \left( -\frac{1}{\tau_1} \right)^{n-2} + \ldots + \lambda_{1n} \right] \frac{e^{-t/\tau_1}}{\left( \frac{1}{\tau_2} - \frac{1}{\tau_1} \right) \left( \frac{1}{\tau_3} - \frac{1}{\tau_1} \right) \ldots \left( \frac{1}{\tau_n} - \frac{1}{\tau_1} \right) + \ldots + (n-1) \text{ similar terms obtained by cyclic permutation of } \tau_1, \ldots, \tau_n } \right]
\]

and analogously

\[
C_i = \frac{D}{V_1} \left[ \left( -\frac{1}{\tau_1} \right)^{n-2} + \lambda_{12} \left( -\frac{1}{\tau_1} \right)^{n-3} + \ldots + \lambda_{ij} \left( -\frac{1}{\tau_1} \right)^{n-j} + \ldots + \lambda_{1n} \right] \frac{e^{-t/\tau_1}}{\left( \frac{1}{\tau_2} - \frac{1}{\tau_1} \right) \left( \frac{1}{\tau_3} - \frac{1}{\tau_1} \right) \ldots \left( \frac{1}{\tau_n} - \frac{1}{\tau_1} \right) + \ldots + (n-1) \text{ similar terms obtained by cyclic permutation of } \tau_1, \ldots, \tau_n } \right]
\]

Generally

\[
C_i = \sum_{j=1}^{n} A_{ij} e^{-t/\tau_j}
\]

(4.39)

Now it is clear that every concentration in every compartment is dependent on the same set of time constants, \(\tau_1, \ldots, \tau_n\), whereas only the coefficients \(A\) differ. Both the time constants and the coefficients are functions of all elementary model parameters (the \(k\)'s and \(V\)'s). The number of exponentials is equal to the number of compartments.

Further it is obvious that when \(t=0\), \(C_i = \frac{D}{V_1}\)

so

\[
\sum_{j=1}^{n} A_{ij} = \frac{D}{V_1}
\]

(4.40)

and that \(C_i = 0\) when \(t=0\)

so

\[
\sum_{j=1}^{n} A_{ij} = 0 \ (i \neq 1)
\]

(4.41)
When the drug is not directly applied into the blood, but to a site from which absorption takes place and when this absorption can be regarded as a first-order process, this can formally be accounted for by introducing a zeroth compartment from which the drug is irreversibly transported to the first (central) compartment. So the set of differential equations 4.18 is extended with

\[
\frac{dQ_0}{dt} = -k_{Ca}C_0 \quad (t=0, Q_0 = D) 
\]

\[
\frac{dQ_1}{dt} = k_{Ca}C_0 - k_{Cel}C_1 - \sum_{i=2}^{n} k_{1i}C_i + \sum_{i=2}^{n} k_{i1}C_i \quad (t=0, Q_1 = 0)
\]

the rest being equal to scheme 4.18. (It should be noted that the decay of the concentration in the zeroth compartment can be derived directly to be

\[
C_0 = \frac{D}{V_0} e^{-t/\tau_a}. \text{We will outline the calculation of } C_1 - C_n
\]

Upon Laplace transformation the problem again is reduced to calculating the determinant of the new set of linear equations (which we call \(\Delta_0\)) and the \(\Delta_1\) derived from it. The relationship between these determinants and those described in the foregoing paragraph can easily be derived and is as follows.

\[
\Delta^0 = (s + \frac{k_{Ca}}{V_0}) \Delta 
\]

(4.43)

and

\[
\Delta_{011} = -\frac{k_{Ca}}{V_1} \Delta_{11} 
\]

(4.44)

\[
\chi_1 = \frac{D}{V_0} \frac{\Delta^0}{\Delta_{011}} = \frac{D}{V_0} \frac{k_{Ca}}{V_1} \frac{\Delta_{11}}{\Delta_{011}} = \frac{D}{V_1 \tau_a} \frac{\Delta_{11}}{(s + \frac{1}{\tau_a}) \Delta} 
\]

(4.45)

where \(\tau_a = \frac{V_0}{k_{Ca}}\) = time constant for absorption

When we express \(C_1\) after oral administration analogously to eq. 4.39 we get
\[ C_i = A_{i0} e^{-t/\tau_a} + \sum_{j=1}^{n} A_{ij} e^{-t/\tau_j} \]  

(4.46)

The time constants \( \tau_j \) are functions of all elementary pharmacokinetic parameters. Only \( \tau_a \) is simply \( \frac{V_0}{k_Ca} \), so the only elementary, model-independent time constant.

For an \( n \)-compartment system equation 4.46 has \( n+1 \) exponential terms. Further it is obvious that when \( t=0, C_i = 0 \) \((i > 1)\).

So \( A_{i0} + \sum_{j=1}^{n} A_{ij} = 0 \)  

(4.47)

The equations 4.18 to 4.47 provide a complete description of mamillary multicompartiment models. The general formulation used allows any number of compartments to be inserted. The main difficulty is the factorization of equation 4.30. When more than two compartments are involved, often numerical methods will be required for this factorization.

**CLEARANCE IN MULTICOMPARTMENT KINETICS**

Since we defined the clearance constant in linear pharmacokinetics as

\[ k_{Cel} = \frac{D}{\int_0^\infty C_1 \, dt} \]  

(4.4)

we can easily express the clearance as a function of time constants and coefficients (in a mamillary model)

\[ k_{Cel} = \frac{D}{\sum_{j=0}^{n} A_{1j} \tau_j} \]  

(4.48)

In case of oral administration \( D \) should be replaced by \( FD \)

This relationship is very useful for calculating the clearance constant, since the \( A \)'s and \( \tau \)'s can all be graphically determined by the well-known method of residuals or can be fitted by a computer program on basis of the plasma concentrations measured as a function of time. The equation of course is valid after both oral and i.v. administration. Eq. 4.48 shows that mistakes can be made in the determination of the clearance constant when not all compartments have been detected. The seriousness of the errors made depends upon the magnitude of the products \( A_j \tau_j \) of the \( A \)'s and \( \tau \)'s that have been overlooked.
DISTRIBUTION VOLUME IN MULTICOMPARTMENT MODELS

In multicompartment models of course every compartment has its own (apparent) volume of distribution. Except for the central compartment, however, these volumes cannot be determined. In case of i.v. administration it follows simply from eq. 4.40

\[ V_1 = \frac{D}{\sum \frac{A_j}{C_{10}}} = \frac{D}{C_{10}} \]  

(4.49)

where \( C_{10} \) is the (extrapolated) drug concentration at time \( t=0 \)

It can easily be derived that the intercepts after oral administration \( A_{ij, oral} \) are simply related to those after i.v. administration:

\[ A_{ij, oral} = \frac{1}{\tau_a} \frac{1}{1 - \frac{\tau_a}{\tau_j}} A_{ij, i.v.} = \frac{1}{\tau_a} \frac{1}{1 - \frac{\tau_a}{\tau_j}} A_{ij, i.v.} \]  

(4.50)

Obviously, after oral administration in multicompartment kinetics the volume of the central compartment may be calculated from

\[ V_1 = \frac{FD}{\sum_{j=1}^{n} \left( 1 - \frac{\tau_a}{\tau_j} \right) A_{1j}} \]  

(4.51)

(note that \( A_{10} \) is excluded from the sum in the denominator)

In multicompartment kinetics the height of the plasma concentration is determined by the dose, the way it is administered, the volumes of all compartments and the rate of exchange between the central and peripheral compartments. These last two factors, the real pharmacokinetic determinants, are reflected in the total apparent volume of distribution, \( V_f \). This volume can be visualized as that volume needed to contain the total amount of drug homogeneously distributed with a concentration equal to the plasma concentration in a multicompartment model where full distribution-equilibrium between the various compartments exists. The total volume of distribution \( V_f \) can be formulated as a function of \( V_1 \) and the time or rate constants of distribution. Suppose at a certain time the total
amount of drug in the compartments 1 \ldots n together to be Q and the amount in the ith compartment to be $Q_i$. When now distribution equilibrium is established this implies for mamillary models:

$$\frac{dQ_i}{dt} = k_{i1} C_1 - k_{i1}^{-1} C_i = 0 \quad (2 \leq i \leq n) \quad (4.52)$$

and

$$\frac{dQ_1}{dt} = -\sum_{i=2}^{n} k_{1i} C_1 + \sum_{i=2}^{n} k_{i1} C_i = 0 \quad (4.53)$$

From eq. 4.52 follows that

$$C_i = \frac{k_{1i}}{k_{i1}} C_1 \quad \text{so} \quad Q_i = \frac{k_{1i} V_i}{k_{i1}} C_1 \quad (4.54)$$

Now

$$Q = V_1 C_1 + \sum_{i=2}^{n} Q_i$$

$$= V_1 C_1 + \sum_{i=2}^{n} \frac{k_{1i} V_i}{k_{i1}} C_1 \quad (4.55)$$

$$C_1 = \frac{Q}{V_1 \left(1 + \sum_{i=2}^{n} \frac{k_{1i} V_i}{k_{i1}}\right)} = \frac{Q}{V_f} \quad (4.56)$$

which means

$$V_f = V_1 \left(1 + \sum_{i=2}^{n} \frac{k_{1i} V_i}{k_{i1}}\right) \quad (4.57)$$

It can be shown that after i.v. administration eq. 4.57 can be formulated as:

$$V_f = \frac{D \sum_{i=1}^{n} A_{1i} \tau_j^2}{\left[\sum_{i=1}^{n} \frac{A_{1j} \tau_j}{2}\right]^2} \quad (4.58)$$

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An analogous expression can be deduced for $V_f$ after oral administration
by the use of eq. 4.50

$$V_{f,oral} = \frac{\text{FD} \sum_{j=1}^{n} A_{1j} \left( \tau_j^2 - \tau_a \tau_j \right)}{\left[ \sum_{j=1}^{n} A_{1j} \left( \tau_j - \tau_a \right) \right]^2} \quad (4.59)$$

From the equations 4.48 to 4.59 it is obvious that determination of the
total clearance and of $V_1$ and $V_f$ can always easily be accomplished,
provided that the plasma concentration is followed long enough. As stated
before serious errors may be made when one or more phases in the plasma
curve are overlooked.

RENAI EXCRETION

The excretion of drugs in urine is governed by four different processes:

a. glomerular filtration: in the glomeruli part of the blood flowing
through the kidneys is ultrafiltrated (about 125 ml filtrate per minute)

b. passive backdiffusion: nearly all the water of the glomerular filtrate is
(partly actively) reabsorbed in the renal tubuli, so that only about
1 ml/min of urine results. Also filtered drug molecules can be passively
reabsorbed during the passage of the primary urine through the tubuli.
This passive backdiffusion is of course pH dependent, since only unionized
drug molecules can be expected to penetrate through the tubular
wall back into the blood.

c. tubular secretion: some drugs are excreted from the capillaries surround-
ing the renal tubuli into the primary urine by an active process (tubular
secretion) analogously to some endogenous ‘waste’ substances. This
process is limited by a certain fixed maximum transport value and
susceptible to competition by various compounds secreted by the same
mechanism.

d. active tubular reabsorption: some drugs are actively reabsorbed from
the primary urine into the capillaries around the renal tubuli; also this
process is analogous to that for endogenous substances (e.g. glucose).
Like tubular secretion this active reabsorption is saturable and suscept-
ible to competition phenomena.
The two last (active) processes are principally non-linear in nature, only at relatively low drug concentrations (low as compared to the dissociation constants between drug and transport mechanisms) the clearance and reabsorption by these processes can be represented by constant, concentration-independent parameters. A more detailed discussion of this matter is given in following chapters. The first two processes (glomerular filtration and passive tubular backdiffusion) in practice can often be summed up to one linear renal clearance constant $k_{\text{Cr}}$, since urinary concentrations have the advantage of being measurable over a relatively long period in case the drug is excreted in urine to a substantial degree. In such a long period fluctuations in urinary pH and urine flow, which may have pronounced influence on the value of the renal clearance constant, usually will average out. A factor that might be of extreme importance in renal excretion of drugs is binding to plasma proteins. Whether or not protein binding will retard renal excretion is primarily determined by the magnitude of the dissociation rate constant of the drug-protein complex, since this will indicate whether the dissociation of the drug from the protein can become the rate limiting step in the excretion process (Rodrigues de Miranda, 1975).

As long as linear pharmacokinetics prevail it is obvious that the rate of excretion of a drug in urine is given by:

$$\frac{dQ_r}{dt} = k_{\text{Cr}} C_p \quad (C_p = \text{plasma concentration}) \quad (4.60)$$

which means that the renal excretion rate as a function of time is a pure reflection of the profile of the plasma concentration curve. Therefore it is possible to obtain time constants for elimination from renal excretion data. Distribution volumes and clearance constants of course cannot be calculated on basis of renal excretion measurements alone. Also the cumulative amount of drug excreted in urine after oral dosage is a measure for the relative bioavailability. However, great care has to be taken in interpreting renal excretion measurements in a quantitative way and it is advisable to draw conclusions only when conditions are standardized as far as possible: constant urine production by waterloading and constant urinary pH by administration of e.g. sodium bicarbonate or ammonium chloride. The renal clearance constant can be obtained from the quotient of the renal excretion rate over a certain period and the average plasma concentration over that period (according to eq. 4.60). On the other hand it can be determined by measuring the total body clearance $k_{\text{Cel}}$ and the total amount of drug excreted unchanged in urine $Q_r,\text{tot}$ on basis of the follow-
ing obvious equation:

\[
\frac{k_{Cr}}{k_{Cel}} = \frac{Q_{r,tot}}{D}
\]  \hspace{1cm} (4.61)

It should be noticed that this equation is also valid in case of oral administration, when the biological availability is principally unknown.

**CHRONIC ADMINISTRATION**

As long as linear pharmacokinetics prevail there exists a simple relationship between equations for the plasma concentration after the first dose and for the plasma concentration after multiple doses. This relation can easily be derived in the following way.

The plasma concentration after the first dose, \( C_1(1) \) is given by

\[
C_1(1) = \sum_{i=0}^{n} A_i e^{-t/\tau_i}
\]  \hspace{1cm} (4.39)

When we denote the dosage interval with \( \Delta t \) the plasma concentration at the end of the first interval is:

\[
C_1(\Delta t) = \sum_{i=0}^{n} A_i e^{-\Delta t/\tau_i}
\]  \hspace{1cm} (4.62)

At \( t=\Delta t \) the second dose is given and from then the plasma concentration obviously is described by

\[
C_1(2) = \sum_{i=0}^{n} (A_i e^{-\Delta t/\tau_i} + A_i) e^{-t/\tau_i}
\]  \hspace{1cm} (4.63)

It should be noted that \( t \) represents here the time elapsed after the last dose (so not from the start of medication). Analogously the plasma concentration after the third dose is given by

\[
C_1(3) = \sum_{i=0}^{n} (A_i e^{-2\Delta t/\tau_i} + A_i e^{-\Delta t/\tau_i} + A_i) e^{-t/\tau_i}
\]  \hspace{1cm} (4.64)

When we switch over to a general formulation, the plasma concentration after the jth dose is:
\[ C_1(j) = \sum_{i=0}^{n} \left( A_i e^{-(j-1)\Delta t/\tau_i} + A_i e^{-(j-2)\Delta t/\tau_i} + \ldots + A_i e^{-t/\tau_i} \right) \]

The preexponential factor (between the brackets) is a geometric series, that can be summed up accordingly

\[ C_1(j) = \sum_{i=0}^{n} \frac{1 - e^{-j\Delta t/\tau_i}}{1 - e^{-\Delta t/\tau_i}} A_i e^{-t/\tau_i} \]

(Once again it is stressed that \( t \) is the time after the jth dose)

Eq. 4.66 is very similar to eq. 4.39, the only difference being that the coefficients \( A_i \) are multiplied by a factor depending on the number of dosages \( j \), the dosage interval \( \Delta t \) and the time constant \( \tau_i \). This factor will always be larger than or equal to 1 and will increase with decreasing \( \Delta t/\tau_i \). In practice this means that phases with a long time constant become more prominent after multiple dosing. It is conceivable that ‘deeper’ compartments that cannot be detected after a single dose, because of the low concentrations associated with it, come into a measureable range after chronic administration, since then deeper compartments are gradually filled up (van Rossum, 1971). The correctness of a model based on single dose experiments therefore can best be verified by measuring plasma decay curves after chronic administration. The increase in the factors \( A_i \) of course is not unlimited; after a certain number of doses a plateau is reached and the plasma concentration will not increase further. From eq. 4.66 it can be seen that this plateau situation is reached when \( j\Delta t \gg \tau_i \). When large differences exist between the various time constants \( \tau_i \) it is possible that pseudo plateau situations are reached. The real plateau is reached when \( j\Delta t \) is very large as compared to the largest time constant involved. Then the following equation holds true:

\[ C_1(p1) = \sum_{i=0}^{n} \frac{1}{1 - e^{-\Delta t/\tau_i}} A_i e^{-t/\tau_i} \]

Once this plateau level has been reached, the profile of the plasma curve over the following intervals of course is the same and the average value of the plateau concentration is given by a very simple equation:
\[ \bar{C}_1(p1) = \frac{1}{\Delta t} \int_0^{\Delta t} C_1(p1) \, dt = \]

\[ = \frac{1}{\Delta t} \int_0^{\Delta t} \sum_{i=0}^{n} \frac{1}{1 - e^{-\Delta t/\tau_i}} A_i e^{-t/\tau_i} \, dt = \frac{1}{\Delta t} \sum_{i=0}^{n} A_i \tau_i \]

(4.68)

By substituting eq. 4.48 we obtain:

\[ \bar{C}_1(p1) = \frac{D}{k_{Cel} \Delta t} \quad \text{or} \quad \bar{C}_1(p1) = \frac{FD}{k_{Cel} \Delta t} \]  

(4.69)

So in linear pharmacokinetics the height of the average plasma plateau level is directly proportional to the dose per interval and inversely proportional to the total body clearance constant and the dosage interval. Eq. 4.69 may also be used for determining the relative bioavailability of various dosage forms after oral administration, since the average plateau concentration after chronic administration is directly proportional to the biological availability F. Further under standardized conditions also the average renal excretion rate during the plateau situation could be used as a measure for this relative bioavailability since it is directly proportional to the average plasma concentration.

Finally, it may be noted that apart from the average plateau concentration, the average concentration during any interval can be calculated analogously

\[ \bar{C}_1(j) = \frac{1}{\Delta t} \int_0^{\Delta t} C_i(j) \, dt = \frac{1}{\Delta t} \sum_{i=0}^{n} \frac{A_i \tau_i}{1 - e^{-j\Delta t/\tau_i}} \]

(4.70)

**CHRONIC ADMINISTRATION IN THE ONE-COMPARTMENT MODEL**

Of special importance for some of the experiments described in chapters 5 and 8 is the chronic oral administration in a one-compartment model. In this case eq. 4.66 can be written as
\[ C_1(j) = \frac{FD}{V_1} \left( \frac{\tau_{el}}{\tau_{el} - \tau_a} \frac{1 - e^{-j\Delta t/\tau_{el}}}{1 - e^{-\Delta t/\tau_{el}}} e^{-t/\tau_{el}} - \frac{1 - e^{-j\Delta t/\tau_a}}{1 - e^{-\Delta t/\tau_a}} e^{-t/\tau_a} \right) \] (4.71)

With regard to this equation the following remarks can be made:

1. No accumulation of drug will occur at all when the dosage interval \( \Delta t \) is large as compared to the time constants for absorption and elimination, \( \tau_a \) and \( \tau_{el} \). Since usually \( \tau_{el} \) is larger than \( \tau_a \) and since in a one compartment system \( \tau_{el} = 1.44 \frac{\tau_a}{\tau_{el}} \), one might use the common formulation that appreciable drug accumulation can only be expected when the dosage interval is smaller than the halflife of the drug.

2. It is interesting to note that in chronic oral administration not only the peak height of the plasma curves during the successive intervals increases until a certain maximum is reached, but that also the time at which the successive peaks are attained (\( t_{max} \)) changes. This can generally be derived by differentiating the equation for \( C_1(j) \) to the time \( t \). This differential quotient is equal to 0 for \( t = t_{max} \)

\[
\left[ \frac{dC_1(j)}{dt} \right]_{t_{max}} = \frac{FD}{V_1} \frac{\tau_{el}}{\tau_{el} - \tau_a} \times \\
\left[ - \frac{1}{\tau_{el}} \frac{1 - e^{-j\Delta t/\tau_{el}}}{1 - e^{-\Delta t/\tau_{el}}} e^{-t_{max}/\tau_{el}} + \right. \\
\left. + \frac{1}{\tau_a} \frac{1 - e^{-j\Delta t/\tau_a}}{1 - e^{-\Delta t/\tau_a}} e^{-t_{max}/\tau_a} \right] = 0 \] (4.72)

This leads to the following expression for \( t_{max,j} \)

\[
t_{max,j} = \frac{\tau_a \tau_{el}}{\tau_{el} - \tau_a} \left[ \ln \frac{\tau_{el}}{\tau_a} + \ln \frac{(1 - e^{-j\Delta t/\tau_a})(1 - e^{-\Delta t/\tau_{el}})}{(1 - e^{-\Delta t/\tau_a})(1 - e^{-j\Delta t/\tau_{el}})} \right] 
\] (4.73)
Clearly \( t_{\text{max}} \) has a limiting value when \( j \Delta t \gg \tau_a \) and \( j \Delta t \gg \tau_{el} \)

\[
t_{\text{max}}(\text{lim}) = \frac{\tau_a \tau_{el}}{\tau_{el} - \tau_a} \ln \left( \frac{\tau_{el} (1 - e^{-j \Delta t / \tau_{el}})}{\tau_a (1 - e^{-\Delta t / \tau_a})} \right)
\] (4.74)

From inspection of these equations it can be seen that during chronic administration \( t_{\text{max}} \) gradually decreases until a certain minimum. In practice, however, this effect might only be measured in extreme cases since usually variations in the time constant for absorption will mask the phenomenon. The peak concentration in any interval of course can be calculated by substituting the value of \( t_{\text{max},j} \) according to eq. 4.73 in the equation 4.71. It may be noted that this analysis clearly shows that the formulas given by Wagner (1971) on page 293 of his textbook are incorrect.

3. In the plateau situation (when \( j \Delta t \gg \) the largest time constant), equation 4.71 obviously reduces to

\[
C_1(\text{pl}) = \frac{FD}{V_1} \frac{\tau_{el}}{\tau_{el} - \tau_a} \left[ \frac{1}{1 - e^{-\Delta t / \tau_{el}}} e^{-t / \tau_{el}} - \frac{1}{1 - e^{-\Delta t / \tau_a}} e^{-t / \tau_a} \right]
\] (4.75)

The average value of \( C_1 \) during an interval in this situation is

\[
\bar{C}_1(\text{pl}) = \frac{1}{\Delta t} \int_0^{\Delta t} C_1(\text{pl}) \, dt = \frac{FD \tau_{el}}{\Delta t V_1} = \frac{FD}{\Delta t \, k_{Cel}}
\] (4.76)

(Note that this result is equal to the general equation 4.69)

4. The equations presented obviously are not valid when \( \tau_a = \tau_{el} = \tau \)

In that case it is easy to show that the equation for the plasma concentration after \( j \) dosages is given by
\[ C_1(j) = \frac{FD}{V_1 \tau} \left[ \Delta t \ e^{-\Delta t/\tau} + 2 \Delta t \ e^{-2\Delta t/\tau} + \ldots \right. \]
\[ \ldots + (j-1) \Delta t \ e^{-(j-1)\Delta t/\tau} + (1 + e^{-\Delta t/\tau}) + \]
\[ + e^{-2\Delta t/\tau} + \ldots \ldots + e^{-(j-1)\Delta t/\tau} \right] e^{-t/\tau} \] (4.77)

The second series in this equation again is a normal geometric one and the first is somewhat different but can be summed up as follows:

\[ \Delta t \ e^{-\Delta t/\tau} + \ldots \ldots + (j-1) \Delta t \ e^{-(j-1)\Delta t/\tau} = \]
\[ = - \frac{d}{d \frac{1}{\tau}} \left( 1 + e^{-\Delta t/\tau} + \ldots \ldots + e^{-(j-1)\Delta t/\tau} \right) = \]
\[ = - \frac{d}{d \frac{1}{\tau}} \left( \frac{1 - e^{-j\Delta t/\tau}}{1 - e^{-\Delta t/\tau}} \right) \]
\[ = \frac{\Delta t\ e^{-\Delta t/\tau} - j\Delta t\ e^{-j\Delta t/\tau} + (j-1)\Delta t\ e^{-(j-1)\Delta t/\tau}}{(1 - e^{-\Delta t/\tau})^2} \] (4.78)

So the plasma concentration after the jth dose can be represented by

\[ C_1(j) = \frac{FD}{V_1 \tau} \left[ \frac{\Delta t\ e^{-\Delta t/\tau} - j\Delta t\ e^{-j\Delta t/\tau} + (j-1)\Delta t\ e^{-(j-1)\Delta t/\tau}}{(1 - e^{-\Delta t/\tau})^2} + \right. \]
\[ + \left. \frac{1 - e^{-j\Delta t/\tau}}{1 - e^{-\Delta t/\tau}} \right] e^{-t/\tau} \] (4.79)

For the plateau situation we now obtain the following equation:

\[ C_1(\text{pl}) = \frac{FD}{V_1 \tau} \left[ \frac{\Delta t\ e^{-\Delta t/\tau}}{(1 - e^{-\Delta t/\tau})^2} + \frac{1}{1 - e^{-\Delta t/\tau}} \right] e^{-t/\tau} \] (4.80)
The average plasma concentration during an interval in the plateau situation can easily be shown to be

\[
\overline{C}_1(\text{pl}) = \frac{1}{\Delta t} \int_0^{\Delta t} C_1(\text{pl}) \, dt = \frac{FD}{\Delta t \, k_{CeI}}
\]

which is equal to the result of equation 4.69.

Figure 4.3 shows a theoretical example of the accumulation profile that can be expected on basis of equation 4.79. The curve is obtained by computer simulation. The validity of equation 4.79 was checked by some other simulations according to equation 4.71, in which values for \( \tau_a \) and \( \tau_{el} \) were inserted that differed only 0.5—1% from each other. The curves obtained then were practically indiscernable from the one given in figure 4.3.

![Figure 4.3](image)

*Simulation of the accumulation of a hypothetical substance according to equation 4.79 (\( V = 6 \, l, \, \tau_a = \tau_{el} = 150 \, \text{min}, \, \Delta t = 50 \, \text{min}, \, D = 500 \, \text{mg} \)). The curve for a substance with the same parameters, but \( \tau_a = 149 \), simulated according to equation 4.71 is practically the same. See text for further explanation.*
REFERENCES

Dost, F.H., Der Blutspiegel, Thieme, Leipzig (1953).
INTRODUCTION

Ibuprofen (2(4-isobutylphenyl) propionic acid)* is a member of a large series of substituted arylacetic acids, which possess analgesic antipyretic and anti-inflammatory activity. A variety of pharmacological test systems have been utilized to show the effectiveness of ibuprofen in animals (Adams et al., 1969; Adams et al., 1970). The spectrum of activity of ibuprofen in these tests appeared to be comparable with that of acetyl-salicylic acid. Toxicological studies in several animal species revealed no serious acute or chronic toxicity (Adams et al., 1969; Adams et al., 1970). Although the drug was shown to have ulcerogenic activity it was established that this was a systemic rather than a local effect and that it was related to the plasma concentration. Extrapolation to the human situation led to the prediction that the risk of gastrointestinal irritation, which is in fact present in all known antiinflammatory drugs, would be relatively small. This has been confirmed in clinical practice. Therapy with ibuprofen up to now has been accompanied by only a low incidence of side-effects. Daily doses up to 1200 mg (in divided portions) appear to be free of serious side-effects (Huskisson et al., 1971). The value of ibuprofen in the treatment of rheumatic disorders has been established in numerous clinical trials (Dick-Smith, 1969; Huskisson et al., 1971). The analgesic and anti-inflammatory effects may be somewhat less than those of aspirin in the usual dosage, but the tolerance usually is better. The antipyretic effect of ibuprofen seems to be quite strong, which makes ibuprofen even suitable for use as a specific antipyretic agent. A recent study concerning primarily the analgesic efficacy of ibuprofen indicates that ibuprofen in episiotomy pain is a useful substitute for acetylsalicylic acid (Bloomfield et al., 1974).

The main metabolites of ibuprofen in man are devoid of pharmacological activity (Adams et al., 1970). In view of the fact that there is a clear correlation between the antiinflammatory activity and the dose administered or the plasma concentration at certain times in rat (Kaiser and Glenn, 1974) a pharmacokinetic characterization of the drug in man seems most useful, since one might expect such a correlation in man too. Adams et al. (1970) and Mills et al. (1973) presented some data concerning the fate of ibuprofen in man. Their results indicate a short halflife and there-

* Brufen®, reg. trademark Boots Pure Drug Company, Nottingham, UK
for a minimal risk of accumulation in dosage regimens of 3 or 4 times daily. Elimination appeared to proceed practically exclusively by formation of metabolites, which in turn are rapidly excreted, about 60% in urine, the rest probably in bile. More systematic measurements of the plasma concentrations after a single oral dose of ibuprofen suggested a half-life of about 2 hr in man (Kaiser and Van Giessen, 1974).

The present studies were undertaken to provide a full pharmacokinetic analysis of ibuprofen in man, including computerfit of plasma concentration data to the appropriate models and calculation of the relevant parameters. Attention has been paid to some biopharmaceutic aspects and to the profile of the plasma concentration curve on repetitive administration. Further the mechanisms of renal clearance involved were analysed in detail.

MATERIALS AND METHODS

Drugs
Ibuprofen (4-isobutylphenylpropionic acid) was kindly supplied by Boots Pure Drug Company, Nottingham, UK. Brufen® tablets, containing 200 mg of ibuprofen, were commercially obtained from O.P.G., Utrecht, The Netherlands.

Drug administration
Three different dosage forms of ibuprofen were employed: Tablets as they are marketed in the Netherlands (Brufen® tablets) containing 200 mg ibuprofen; gelatine capsules containing 200 mg ibuprofen pure substance and solutions, freshly prepared by dissolving 400 mg ibuprofen in 3 ml of 10% NaHCO₃ solution and diluting with water to 200 ml. Tablets and capsules were taken together with about 200 ml of water.

The volunteers participating in the study were all young, healthy students who did not receive any other medication during and for a period of at least two weeks prior to the trials. None of them had ever suffered from liver or kidney function disturbances. The subjects fasted overnight prior to the ingestion of the drug at 9.00 a.m. and for an additional 3 hrs after drug intake. At regular times after drug administration 7 to 10 blood samples of about 5 ml were taken from a fore arm vein and collected in heparinized tubes. For at least 30 hrs from the time of drug intake all volunteers collected samples of every aliquot of urine they voided, after recording the time and the total volume of production. The various
volunteers who participated twice or thrice in this study had at least a one-week interval between two experiments. One subject took two Brufen® tablets three times daily (at 1.00, 9.00 and 17.00 hrs exactly) for a period of 14 days. In this experiment samples were taken throughout the period, frequently after the first and the last dose and in the period in between two or three samples every other day. The blood samples were centrifuged, plasma was stored in a deepfreeze (−20°C) until assay. The erythrocytes were mostly discarded. Only in some experiments also the erythrocytes were analysed for their ibuprofen content.

**Preparation of samples and gaschromatographic analysis**
As described in chapter 2.

**Protein binding**
The binding of ibuprofen to albumin was determined by equilibrium dialysis in the apparatus described by Rodrigues de Miranda (1975). In the protein compartment a solution of 2.8% bovine serum albumin in phosphate buffer pH 7.4 with 0.9% NaCl added was present. The other compartment contained ibuprofen in concentrations ranging from 20 to 75 mg/l in an equal volume of the same solvent. Dialysis was performed at 37°C for 20 hrs. During this equilibration process no change in protein concentration occurred. After 20 hrs equilibrium was reached as was confirmed by pilot experiments in which ibuprofen was added alternatively to the protein free and to the other compartment. Ibuprofen analysis at both sides of the membrane was performed as described in chapter 2.

**RESULTS AND DISCUSSION**

**The profile of the plasma curve**
Ibuprofen is rapidly absorbed and its concentration in plasma thereafter declines to negligible levels within 10 hrs after administration of the three dosage forms to the healthy volunteers in this study. Some typical examples of plasmacurves together with renal excretion data are given in fig. 5.1. The profile of the plasma curves can satisfactorily be described according to the open one compartment model (cf. chapter 4). With the aid of the computer program Farmfit the plasma concentration data were fitted to the appropriate equation:

\[
C = A \left( e^{-t/\tau_{el}} - e^{-t/\tau_a} \right) \quad (5.1)
\]
Figure 5.1
Plasma curves and renal excretion after oral administration of ibuprofen (400 mg).
where \[ A = \frac{FD}{V} \frac{\tau_{el}}{\tau_{el} - \tau_a} \]  

(5.2)

Table 5.1 shows a compilation of the kinetic parameters obtained by this fitting procedure. It should be emphasized that the distribution volume, \( V \), and the clearance constant, \( k_{Cel} \), cannot be determined in absolute terms after oral administration, since the biological availability, \( F \), is not known. Therefore at least part of the variability in the values estimated for the volume of distribution and for the clearance constant will be due to differences in \( F \).

**TABLE 5.1**  
Pharmacokinetic parameters for ibuprofen after oral administration (D = 400 mg).

<table>
<thead>
<tr>
<th>Subject</th>
<th>V/F</th>
<th>( k_{Cel}/F ) (ml/min)</th>
<th>( \tau_{el} ) (min)</th>
<th>( t_{1/2} ) (min)</th>
<th>( \tau_a ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SOLUTION(^a)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JE (23,91,186)</td>
<td>6.1</td>
<td>39</td>
<td>156</td>
<td>108</td>
<td>0.7</td>
</tr>
<tr>
<td>LH (23,66,176)</td>
<td>6.0</td>
<td>41</td>
<td>146</td>
<td>101</td>
<td>2.3</td>
</tr>
<tr>
<td>VR (20,67,180)</td>
<td>4.8</td>
<td>31</td>
<td>158</td>
<td>110</td>
<td>1.1</td>
</tr>
<tr>
<td>WS (22,78,187)</td>
<td>6.2</td>
<td>37</td>
<td>166</td>
<td>115</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>CAPSULE(^a)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JA (22,70,181)</td>
<td>7.6</td>
<td>49</td>
<td>156</td>
<td>108</td>
<td>8.9</td>
</tr>
<tr>
<td>DB (20,80,185)</td>
<td>8.6</td>
<td>53</td>
<td>160</td>
<td>111</td>
<td>15.3</td>
</tr>
<tr>
<td>GB (20,65,178)</td>
<td>9.2</td>
<td>54</td>
<td>170</td>
<td>118</td>
<td>5.1</td>
</tr>
<tr>
<td>BK (25,60,173)</td>
<td>5.7</td>
<td>38</td>
<td>153</td>
<td>106</td>
<td>2.7</td>
</tr>
<tr>
<td>HL (23,76,183)</td>
<td>6.4</td>
<td>36</td>
<td>178</td>
<td>124</td>
<td>6.1</td>
</tr>
<tr>
<td>TS (20,67,180)</td>
<td>8.3</td>
<td>53</td>
<td>155</td>
<td>108</td>
<td>4.7</td>
</tr>
<tr>
<td><strong>TABLET</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (23,81,184)</td>
<td>4.6</td>
<td>36</td>
<td>126</td>
<td>87</td>
<td>46</td>
</tr>
<tr>
<td>JB (23,67,176)</td>
<td>8.6</td>
<td>51</td>
<td>167</td>
<td>116</td>
<td>75</td>
</tr>
<tr>
<td>JB(^4)</td>
<td>5.7</td>
<td>35</td>
<td>162</td>
<td>112</td>
<td>30</td>
</tr>
<tr>
<td>JE (23,91,186)</td>
<td>11.0</td>
<td>72</td>
<td>152</td>
<td>105</td>
<td>88</td>
</tr>
<tr>
<td>JH (25,57,174)</td>
<td>7.0</td>
<td>46</td>
<td>153</td>
<td>107</td>
<td>29</td>
</tr>
<tr>
<td>LH (23,66,176)</td>
<td>7.4</td>
<td>49</td>
<td>151</td>
<td>105</td>
<td>57</td>
</tr>
<tr>
<td>JHo (22,75,189)</td>
<td>8.9</td>
<td>51</td>
<td>174</td>
<td>121</td>
<td>16</td>
</tr>
<tr>
<td>MR (21,54,167)</td>
<td>7.1</td>
<td>46</td>
<td>155</td>
<td>108</td>
<td>10</td>
</tr>
<tr>
<td>VR (20,67,180)</td>
<td>8.1</td>
<td>52</td>
<td>163</td>
<td>113</td>
<td>12</td>
</tr>
<tr>
<td>WS (22,78,187)</td>
<td>11.5</td>
<td>70</td>
<td>172</td>
<td>119</td>
<td>26</td>
</tr>
</tbody>
</table>

1. Numbers in parentheses represent age (yr), bodyweight (kg) and height (cm); all subjects are male, except MR.
2. Relative error in \( \tau_a \) is high; upper limit is about 3 min.
3. These data are taken from the study of chapter 7.
4. Parameters determined after chronic administration (400 mg 3 dd for 2 weeks).
Absorption rate
For all 3 dosage forms absorption was a rapid process, although in case of the commercial tablets in a few cases an unusually long time constant for absorption was observed. These seem to be exceptions, however, caused by individual factors rather than by the drug formulation. Although all curves were analysed for the possible occurrence of a lagtime, introduction of this additional parameter did not give any significant improvement of the fit in most cases. Only when commercial tablets were administered, in 3 out of 10 participants (AA, WS, JB) a lagtime varying from 10 to 20 minutes appeared to be desirable in the sense that a better fit resulted with this additional parameter.

The absorption rate seems to be dependent on the pharmaceutical formulation. The average time constants for absorption of tablet, capsule and solution are in the order of 20, 5 and 1 minutes respectively. These differences certainly are not unexpected. Since a drug has to be dissolved before it can be absorbed it is quite understandable that administration of the drug in solution warrants the highest absorption rate. The advantage of gelatin capsules over the commercial tablets might be that the capsules release the drug more easily in a finely dispersed form, resulting in a higher dissolution rate of the drug in the gastrointestinal fluid. From a practical point of view, however, also ease of storage and dispensing of the drug formulation should be considered. Taking these factors into account we therefore tend to conclude that differences in absorption rate do not justify preference of capsules or solutions over tablets especially since even for tablets plasma levels near to maximal are reached as a rule within 30 minutes.

Elimination rate
The rate of elimination of ibuprofen from plasma is quite high. The halflife \((t\frac{1}{2})\), which is a significant parameter in this respect, varies roughly from 1.5 to 2 hrs. The halflife is determined by two fundamental pharmacokinetic parameters: the volume of distribution representing the volume over which the drug can be imagined to be distributed on basis of the measured plasma concentration and the clearance constant which is a measure for the efficiency of the clearing organs (liver and kidney especially) in eliminating the particular drug from the body.

Volume of distribution and biological availability
As was stated before after oral administration the volume of distribution cannot be determined absolutely, since the biological availability is essen-
tially unknown. Therefore in table 5.1 the quotient $V/F$ is given, which obviously can be calculated according to:

$$\frac{V}{F} = \frac{D}{A} \frac{\tau_{el}}{\tau_{el} - \tau_a}$$

This implies that the real volume distribution, $V$, may be smaller than this value, since the biological availability by definition is maximally equal to unity. It also implies that when various formulations are administered to the same volunteer the lowest estimation of $V/F$ is the best approximation of the actual volume of distribution, since it corresponds with the highest biological availability. The differences found in the values $V/F$ for the volunteers WS, JE, VR and LH after administration of tablets and solution can easily be explained by assuming the biological availability of the tablet form to be lower than that of the solution. The ratio $F_{tablet} : F_{solution}$ should be 0.54; 0.55; 0.59 and 0.81 for the four volunteers respectively. Additional evidence in favour of this assumption lies in the fact that the time constant for elimination is not significantly different after tablets and solution and that the distribution volume or the clearance constant hardly could change so much in a few weeks time without other noticeable signs. Furthermore, when the experiments were repeated after several months roughly the same differences in height of the plasma levels were encountered. Capsules seem to be comparable with tablets in this respect. In conclusion, our data suggest that the biological availability of the tablet (and capsule) form is quite variable, being comparable to that of solutions in some cases, but definitely lower in other cases.

It should be borne in mind that the volunteers in this study were fasting overnight prior to drug ingestion; this together with the fact that they were all young and healthy restricts of course generalization of the conclusions.

The availability of the solution most probably will be nearly complete; the actual volume of distribution according to the open one-compartment model therefore seems to be in the order of 5 to 7 liters (0.06 to 0.08 liters per kilogram bodyweight), since still smaller volumes would be very unusual. Roughly this estimation coincides with the blood volume. However, since we know that the concentration of ibuprofen in the erythrocytes is much lower than in plasma and that the drug in plasma is highly protein bound (see below) it is reasonable to think of blood, extracellular and possibly also intracellular water as the phases over which ibuprofen is distributed. That the pharmacokinetic distribution volume is
only about 50% of the volume of the extracellular fluid is then directly related to the strong protein binding, causing disproportionately high plasma levels.

**Clearance**

Since the clearance constant \( k_{Ce} \) equals the quotient \( V/\tau_{el} \) it is obvious that the estimation of this parameter is also dependent on the biological availability. The same discussion as given above for the distribution volume therefore can be applied to the clearance constant. When the values for the relative clearance constants \( k_{Ce}/F \) for the subjects who took both tablets and solution are corrected for the differences in \( F \), as argued above, it appears that the clearance constant is independent of drug formulation. Another result of course would be hardly feasible. Taking these considerations into account, the best estimate for the value of the clearance constant is in the range of 30 to 50 ml/min. The clearance of ibuprofen occurs almost exclusively by metabolic transformation. The contribution of renal excretion is negligible for all practical purposes (see below). Since we are dealing with plasma clearance and since the blood cells contain only minor amounts of drug we can compare the clearance constant with the plasma-flow through the liver, which is approximately 750 ml/min. Assuming that the metabolism predominantly takes place in the liver, one might calculate that in the liver only about 4-7% of the drug is extracted from the plasma passing through. This does not seem to be a very efficient process and a retarding effect by the strong binding to albumin in the plasma is very well possible. Also the very low renal clearance may be caused by this binding, but on the other hand substantial reabsorption may be an important factor here. The fact that the protein binding seems to inhibit renal excretion to a much higher degree than metabolic conversion might be due to differences in the average transit times. The hepatic transit time can be expected to be longer than the renal cortical transit time, so the dissociation of the drug-protein complex will be more easily rate limiting in the kidney than in the liver. Evidence in favour of this assumption is obtained by comparing the bloodflow through the kidneys and the liver per equal amount of tissue. For the kidneys this is as high as 400 ml/min per 100 ml of tissue, whereas for the liver a value of about 40 ml/min per 100 ml of tissue is found, so a factor 10 lower (Mapleson, 1963). Furthermore the vascular system becomes quite diffuse in the liver, thereby allowing even protein bound drug to leave the blood vessels.

The low metabolic clearance constant implies that no significant first pass effect can occur, although the rate of elimination of the drug from
plasma is rather high (see also chapters 8 and 10B). The fact that despite the low clearance constant a high elimination rate is found is a direct consequence of the small apparent volume of distribution, to which the rate of elimination is inversely proportional.

**Renal excretion**

The relevant renal excretion data are summarized in table 5.2. Obviously only a negligible amount of drug is excreted unchanged by the kidney whereas a more substantial amount is found in urine in the form of its glucuronide. Some examples of the urinary excretion rate both for free and for glucuronidated ibuprofen are given in figures 5.1 and 5.2. Although the renal excretion of unchanged ibuprofen is practically negligible the renal clearance appeared to be a highly interesting process. This is demonstrated in fig. 5.3 where the renal excretion rate is plotted as a function of the average plasma concentration over the period of excretion.

**TABLE 5.2**

Cumulative renal excretion in % of dose (Qₜₕ%) of unchanged ibuprofen and ibuprofen-glucuronide after oral administration of ibuprofen (D=400 mg).

<table>
<thead>
<tr>
<th>Subj</th>
<th>Qₜₕ % (unch.)</th>
<th>Qₜₕ % (gluc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0.71</td>
<td>19.25</td>
</tr>
<tr>
<td>JB</td>
<td>0.35</td>
<td>7.65</td>
</tr>
<tr>
<td>JE</td>
<td>0.81</td>
<td>–</td>
</tr>
<tr>
<td>JH</td>
<td>0.55</td>
<td>12.80</td>
</tr>
<tr>
<td>LH</td>
<td>0.64</td>
<td>–</td>
</tr>
<tr>
<td>JHo</td>
<td>0.27</td>
<td>–</td>
</tr>
<tr>
<td>VR</td>
<td>1.39</td>
<td>–</td>
</tr>
<tr>
<td>WS</td>
<td>2.50</td>
<td>–</td>
</tr>
<tr>
<td>CAPSULE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JA</td>
<td>0.83</td>
<td>13.90</td>
</tr>
<tr>
<td>DB</td>
<td>1.45</td>
<td>8.85</td>
</tr>
<tr>
<td>GB</td>
<td>0.35</td>
<td>10.00</td>
</tr>
<tr>
<td>BK</td>
<td>0.80</td>
<td>9.25</td>
</tr>
<tr>
<td>HL</td>
<td>2.06</td>
<td>16.20</td>
</tr>
<tr>
<td>TS</td>
<td>0.43</td>
<td>10.90</td>
</tr>
</tbody>
</table>

– not determined.
Renal excretion of free and glucuronidated ibuprofen in a volunteer who received 400 mg ibuprofen (capsule). Note the much larger excretion of the glucuronide.

The profile of these graphs suggests at least two mechanisms governing ibuprofen excretion, viz. glomerular filtration and tubular secretion. If only glomerular filtration would occur the plots in fig. 5.3 should be straight lines passing through the origin. The profile that is found experimentally is in accordance with the superposition of tubular secretion upon glomerular filtration. Tubular secretion usually is an active process, which is saturated when the supply of substrate exceeds certain limits. The simultaneous occurrence of glomerular filtration and tubular secretion can be formulated as follows:

\[
\frac{dQ_r}{dt} = \left[ k_{Cr} C_p + \frac{T_M}{K_T + C_p} \right] C_p
\]  \hspace{1cm} (5.4)

where

\[
\frac{dQ_r}{dt} = \text{rate of urinary excretion (e.g. in \(\mu g/min\))}
\]
\[ k_C^1 = \text{clearance constant for glomerular filtration (combined with passive reabsorption; e.g. in ml/min)} \]

\[ T_M = \text{tubular transport maximum = the amount of drug that can be secreted maximally per unit of time (e.g. in mg/min)} \]

\[ K_T = \text{Michaelis Menten constant for the drug with respect to the secretion mechanism (e.g. in mg/ml or mg/l)} \]

\[ C_p = \text{plasma concentration} \]

The two processes can be differentiated as exemplified in the figure. One makes use of the fact that tubular secretion becomes saturated at high plasma levels where \( C_p \gg K_T \). Then the renal excretion rate is given by:

\[
\frac{dQ_f}{dt} = k_C^1 C_p + T_M
\]

(5.5)

**Figure 5.3**

Renal excretion rate of ibuprofen as a function of the average plasma concentration over each interval. Note the non-linear curves, indicating the occurrence of tubular secretion. The graphs can be analysed as discussed in the text and give an impression of the relative contribution of filtration (GF) and tubular secretion (TS) to the overall excretion. The plasma concentration at half-maximal tubular secretion represents the Michaelis-Menten constant of the secretion mechanism \((K_T)\).
so that the excretion rate at high plasma levels becomes a linear function of the plasma concentration. The straight line is shifted with respect to the origin by the amount $T_M$. So a line through the origin parallel to the final straight part of the excretion curve represents the linear part of renal excretion (filtration). By subtracting this line from the experimental curve the process of tubular secretion is found. $K_T$ is equal to the plasma concentration at which the tubular secretion rate is half-maximal and can be found experimentally by interpolation. Obviously also $T_M$ is easily accessible (figure 5.3). Unfortunately the values found for these parameters ($K_T = 2-4$ mg/l and $T_M = 2-4 \mu g/min$) are purely operational, since one cannot be sure which plasma concentration is meant with $C_p$ in equations 5.4 and 5.5. We derived the parameters for the case that $C_p$ is the total plasma concentration, comprising both free and protein-bound drug. This, however, is a questionable assumption, since protein binding certainly will retard the rate of tubular secretion (Rodrigues de Miranda, 1975). The degree of this effect will be dependent on the rate of dissociation of the drug-protein complex. The same holds true in fact for the glomerular filtration process. Taking into account that in the concentration range studied about 99% of the drug in plasma is protein bound we can conclude that the estimated value for the clearance constant for glomerular filtration may be even a factor 100 too low, when no dissociation of the drug from the protein occurs at all during the process of filtration. The actual clearance constant will be somewhere in between and cannot be calculated on basis of the present data alone.

The fact that strong indications for tubular secretion of ibuprofen are found is not surprising. Despopoulos (1965) summarized molecular characteristics required for tubular secretion. One of these is the occurrence of a free, ionized carboxyl group as it is present in ibuprofen. However, the secretory mechanism has only a low capacity for ibuprofen and is easily saturated. This may be caused by the fact that other prerequisites are lacking, for instance an amino function which seems to enhance tubular secretion strongly. As far as the influence of protein binding is concerned it should be realized that such binding counteracts the appearance of tubular secretion. Characteristic for the binding is the fact that at low drug concentrations the fraction of bound drug is relatively higher, so the amount of drug directly available for excretion or secretion is relatively smaller than at high levels. This implies that at low plasma concentrations the renal excretion rate is decreased by protein binding below the theoretical level that would be obtained in the absence of protein binding. Such an effect is opposite to the one measured, so one may conclude that
on basis of free drug concentrations the appearance of tubular secretion would be more pronounced.

Since only very minor amounts of ibuprofen are excreted in urine unchanged, there will be no need for dosage adjustment in renal function impairment. Furthermore, competition by other drugs for the tubular secretion mechanism can never be significant in that quantitatively only negligible amounts of ibuprofen are involved in this process. On the other hand, ibuprofen might give rise to substantial inhibition of tubular secretion of other drugs that are secreted by the same mechanism to a higher amount, since the degree of inhibition is primarily determined by the ratio of the plasma concentration over its $K_T$ value. That up to now no serious interactions in this respect are reported may be caused by the fact that such drugs are rare or even not existing.

**Metabolites in urine**

Table 5.2 shows that approximately 10–20% of the dose administered is excreted in urine as ibuprofen-glucuronide. See also fig. 5.2. The occurrence of this ester-type glucuronide is not unexpected since conjugation to

![Renal excretion rate of two presumable metabolites of ibuprofen.](image)

**Figure 5.4**

*Renal excretion of two presumable metabolites of ibuprofen. Mass spectrometric analysis of the compounds indicated a structural similarity to ibuprofen and suggested oxidation of the isobutyl group.*
glucuronic acid is a common metabolic pathway for carboxylic acids. Gas-chromatograms of urine extracts obtained usually contain two other peaks with retention times about 3 and 6 times that of ibuprofen. The increase and subsequent decrease of the height of these peaks was reminiscent of what could be expected for metabolites of ibuprofen (fig. 5.4). Mass spectra obtained from silylated urine extracts in the GCMS LKB 9000 were in accordance with the structure of the two urinary metabolites as proposed by Adams et al. (1967). Roughly estimated the total amount of these metabolites excreted was about 1—2% of the dose, but it cannot be excluded that more substantial amounts are excreted as glucuronide. As far as the large remaining part of the dose, not accounted for, is concerned one may think of biliary excretion as a major pathway of elimination, especially for metabolites. Indications for this can be found in literature (Adams et al., 1970; Mills et al., 1973).

**Protein binding**
Under the circumstances as described and in the concentration range applied (20—75 mg/l) the binding of ibuprofen to bovine serum albumin appeared to be more than 98%. At plasma concentrations below 20 mg/l the theoretical binding percentage is even higher. On the basis of our data we estimated the association constant of the drug-protein complex $K_{ass}$ to be in the order of $10^5 - 10^6$ l/mol. This agrees reasonably with the value of $10^5$ l/mol that Mills et al. (1973) found for the association of ibuprofen to human plasma. In practice these binding data imply that at usual (therapeutic) concentrations the drug in plasma is nearly completely protein bound (binding percentage more than 98%). As discussed above this high protein binding probably will contribute to the small volume of distribution and to the relatively small renal and metabolic clearance. The combination of high plasma levels (caused by small distribution volume) and the high degree of binding to plasma proteins might be expected to give rise to serious interactions with other drugs at the level of protein binding. Up to now, however, no data are available in this respect, nor have serious clinical consequences been reported.

**Erythrocytes**
Figure 5.5 shows for two volunteers the concentration of ibuprofen in the erythrocytes as compared to the plasma levels. The concentration in erythrocytes appears to be slightly less than 10% of that in plasma, but equilibrium seems to be established rapidly, since the lines are reasonably parallel, so the ratio is constant. Several factors might contribute to the
finding that erythrocyte concentrations are so much lower than plasma concentrations. First of all there is a difference in pH: the pH of plasma is 7.4 whereas that of erythrocyte cystosol is 7.25. The $pK_a$ of ibuprofen is 4.4 as we determined by differential potentiometric titration similar to the method of Janssen et al. (1970). This pH difference accounts for about 30% lower concentrations in erythrocytes than in plasma. The second and important factor is the possible difference in protein binding: ibuprofen is highly bound to plasma protein, but probably much less to cellular proteins although no exact data are available. Finally, there is a source of error in the experimental method. After centrifugation erythrocytes remain somewhat contaminated with plasma. When this contamination would mount up to for instance 10%, the only justified conclusion would be that erythrocytes contain not more than negligible amounts of ibuprofen, since the levels found would be due to contamination with plasma. This last situation cannot be excluded in the light of the experimental procedures.

Figure 5.5
Concentration of ibuprofen in erythrocytes as compared with the concentration in plasma. Note the parallel course, which indicates rapid equilibration.
Repeated administration
Mills et al. (1973) presented evidence that neither ibuprofen itself nor its known metabolites had a tendency to accumulate in plasma in man (and in several animal species) after repetitive administration during 14 days. For ibuprofen this is not surprising in view of its short half-life (1.5—2 hrs) with respect to the usual dosage interval (8 hrs). As was shown in chapter 4 the average steady state plasma concentration after chronic administration is given by:

$$\bar{C}_p = \frac{FD}{\Delta t k_{Cel}}$$

Inserting the values found for ibuprofen in this study one can calculate that the average steady state concentration in a dosage regimen of 400 mg thrice daily is about 20 mg/l, which is hardly above the average concentration after a single 400 mg dose. This also implies that such dosage regimens are subject to very pronounced fluctuations in the plasma concentration.

Figure 5.6
Plasma concentration during repeated administration (400 mg, 3 dd) of ibuprofen. Practically no accumulation occurs and the plasma level is very fluctuating. The curve is generated on basis of the parameters obtained after a single dose and gives a reasonable adaptation to the experimental data.
All of these predictions are confirmed experimentally as exemplified in figure 5.6 where the plasma concentration during chronic administration to a volunteer in a regimen of 400 mg thrice daily (every 8 hrs) is given. Although the profile is exactly as expected, which also confirms that the open one-compartment model yields an adequate description of ibuprofen kinetics, serious questions remain open for discussion. For instance, the question of how it is possible that these fluctuating plasma levels can lead to apparently satisfactory pharmacological effects. One possible explanation for the discrepancy between plasma levels and presumed effect is that only temporarily high levels are needed to regulate one or another disturbed process, which then automatically remains in order for a longer period of time. Another possibility would be that the plasma concentration is not a good reflection of the effective drug concentration at the site of action. One might think for instance of concentration of ibuprofen in leucocytes or in the synovial fluid as a better guide for the efficiency of the drug, since these compartments probably are more directly connected with the site of action of the drug.

REFERENCES

INTRODUCTION

In the preceding chapter the pharmacokinetics of ibuprofen in man have been discussed. Since ibuprofen contains an asymmetric carbon atom, the question arose if there exists a difference in pharmacological activity and pharmacokinetic behaviour between the two optical isomers. As far as the activity is concerned, it is quite common that there exists a difference between optical isomers (see for instance Rodrigues de Miranda, 1975). Also in the field of antiinflammatory phenylacetic acid derivatives, analogous to ibuprofen, it has been found that the dextro-isomers are often much more potent than the levo-isomers (Shen, 1967). However, in case of ibuprofen, Adams et al. (1967) reported that no difference exists in the antiinflammatory potency of the two isomers in the guinea pig ultraviolet erythema test. Interestingly they also found that the principal urinary metabolites of racemic ibuprofen in man were dextrorotatory. These facts might indicate an isomerisation step from the levo- to the dextro-isomer of ibuprofen in vivo. Support for this idea is given by the observation that after administration of the pure levo-isomer the urinary metabolites were dextrorotatory, although to a lesser extent than after the dextro-isomer itself indicating that only incomplete inversion takes place (Mills et al., 1973). Finally, after completion of our study, Van Giesen and Kaiser (1975) published a method for differential measurement of the optical isomers of ibuprofen in plasma or urine. Applying this method to the analysis of plasma samples after administration of the racemic mixture they found the levo-enantiomer to disappear substantially more rapidly from plasma than the dextro-isomer. As a matter of fact on the basis of these observations, one would expect a biphasic elimination of racemic ibuprofen. In our previous study, however, we did not find any indication for that. Therefore we decided to follow the plasma concentration and renal excretion after administration of the pure dextro- and levo-enantiomers as well as the racemic ibuprofen in the same volunteers, in order to see whether the pharmacokinetics of the individual isomers sum up to the monoexponential decay found for the racemate.
MATERIALS AND METHODS

Drugs, reagents, preparation of urine and blood samples, glucuronidase treatment, gaschromatography were the same as described in chapters 2 and 5, except for the following additions.

l(−)—l-phenylethylamine and d(+)—l-phenylethylamine were obtained from Aldrich, Milwaukee, Wisconsin, USA.

A gift of reference amounts of the d- and l-isomers of ibuprofen from Boots Pure Drug Company (Nottingham, England) is gratefully acknowledged.

Resolution of ibuprofen

To a solution of racemic ibuprofen in hot acetone an equimolar amount of l(−)—l-phenylethylamine in acetone was added (cf. Adams et al., 1967). The precipitating phenylethylammonium salt was collected in four about equal fractions. The first fraction of the salt was recrystallized twice from acetone. A melting point of 160—162°C was obtained. The salt was dissolved in HCl 2N and this solution was extracted with n-hexane. After crystallization the resulting ibuprofen (mp 49.5—50.5) appeared to be sufficiently pure dextro-isomer with a specific rotation $[\alpha]^D_{25} = 53.6^\circ$ (in ethanol). From the third and fourth fraction, which were of course already enriched with l(−)-isomer, ibuprofen was isolated. This was dissolved in hot acetone and mixed with an equimolar amount of d(+)—l-phenylethylamine in acetone. Again the precipitate was collected in four fractions. The first fraction was crystallized from acetone, then from toluene and finally from chloroform. The resulting melting point was 149—151°C. From this salt ibuprofen was isolated and then crystallized from petroleum ether. The specific rotation of this ibuprofen fraction (mp 50—51°C) in ethanol was $[\alpha]^D_{25} = -55.5^\circ$, indicating sufficiently pure levo-isomer.

The overall yield in this resolution procedure was about 8% for both isomers, calculated on basis of total amount of racemic starting material.

Drug administration

Four volunteers participated in this study. At the first day of the series of experiments they received 200 mg of racemic ibuprofen; one week later two of them received 200 mg of the dextro-isomer and the other two 200 mg of the levo-isomer; after another week each volunteer got 200 mg of the other isomer and two weeks later all of them ingested a control dose of 200 mg of the racemate. In the last experiment only 5 blood samples were drawn in order to confirm that the volunteers showed the same pharma-
cokinetic pattern as in the first experiment. In the other experiments 10 blood samples were taken over a period of 9 hrs after drug administration. The conditions were the same as described in chapter 5.

The dosage form in all cases was the same and consisted of 200 mg ibuprofen (or ibuprofen-isomer) dissolved in 3 ml NaHCO₃ 10% solution, diluted with water to 200 ml.

RESULTS AND DISCUSSION

The pharmacokinetic parameters obtained are summarized in table 6.1 and figure 6.1 shows as an example for one of the volunteers the plasma levels and the urinary excretion data after ingestion of racemic, dextro- and

**TABLE 6.1**

Pharmacokinetic parameters after oral administration of ibuprofen isomers (D=200 mg).

<table>
<thead>
<tr>
<th>Subject</th>
<th>isomer</th>
<th>Vₚ/F (l)</th>
<th>kₚ/CₚF (ml/min)</th>
<th>τₑₚ (min)</th>
<th>τₓ/₂ (min)</th>
<th>τₐ² (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JE (24,90,186) +</td>
<td>6.3</td>
<td>45</td>
<td>140</td>
<td>97</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>- -</td>
<td>5.5</td>
<td>30</td>
<td>180</td>
<td>125</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>± ±</td>
<td>5.9</td>
<td>37</td>
<td>160</td>
<td>111</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>C C</td>
<td></td>
<td></td>
<td></td>
<td>106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH (24,66,176) +</td>
<td>7.7</td>
<td>51</td>
<td>150</td>
<td>104</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>- -</td>
<td>4.1</td>
<td>26</td>
<td>160</td>
<td>111</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>± ±</td>
<td>5.6</td>
<td>37</td>
<td>151</td>
<td>105</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>C C</td>
<td></td>
<td></td>
<td></td>
<td>109</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VR (21,69,180) +</td>
<td>6.3</td>
<td>55</td>
<td>115</td>
<td>80</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>- -</td>
<td>4.5</td>
<td>24</td>
<td>186</td>
<td>129</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>± ±</td>
<td>5.0</td>
<td>33</td>
<td>152</td>
<td>106</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>C C</td>
<td></td>
<td></td>
<td></td>
<td>110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WS (23,79,187) +</td>
<td>8.7</td>
<td>55</td>
<td>159</td>
<td>110</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>- -</td>
<td>5.7</td>
<td>37</td>
<td>153</td>
<td>106</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>± ±</td>
<td>7.0</td>
<td>45</td>
<td>156</td>
<td>108</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>C C</td>
<td></td>
<td></td>
<td></td>
<td>101</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Numbers in parentheses represent age (yr), bodyweight (kg) and height (cm); all subjects are male.
2. The relative error in τₐ in high; upper limit about 3 min.
3. "C" refers to a control experiment where only half-life of the racemate was measured.
levorotatory ibuprofen. The data reveal some interesting differences in the pharmacokinetic behaviour of the enantiomers. In any case, the elimination half-life of the dextro-isomer tends to be smaller than that of the levo-isomer, whereas the half-life of racemic mixture has a value in between. The differences, however, are only in 2 of the volunteers significant. The theoretical plasma profile for the racemate, as expected on basis of the average of the separate curves for the enantiomers, is in close agreement with the experimental results. In principle the elimination of the racemate proceeds in a biphasic way, which is not detected experimentally because of the relatively small difference in elimination rate of the optical isomers. More pronounced differences are encountered in the values for $V/F$ and $k_{Cel}/F$ as they are given in table 6.1. One might expect some random variation to occur in these parameters since all drug formulations have been given orally. However, the variations are not arbitrary, but systematic differences occur. In any one of the volunteers the dextro-isomer exhibits a larger apparent volume of distribution and a larger clearance constant than the levo-isomer. As should be expected when dealing with real, fundamental differences in these parameters, the values for the racemic ibuprofen are about the average of the values for the enantiomers separately. This evidently suggests that the measured differences are not caused by variable biological avail-

**Figure 6.1**

*Plasma curves of ibuprofen and cumulative renal excretion of ibuprofen and its glucuronide after oral administration of the separate isomers and of the racemate.*
ability but are due to differences in the intrinsic pharmacokinetic behaviour of the substances. It may be noted, furthermore, that absorption from the drug solution applied was extremely rapid and started without delay. The estimated time constants for absorption are in all cases smaller than 0.6 minutes and no lag-time occurs. Provided that the absorption can be regarded as a linear process, this implies that absorption is completed within a few minutes. In that case it does not make sense that the bioavailability of the dosage form would not be about unity. But even when absorption is not a linear process, which means that \( \tau_a \) would only be an operational parameter, it still can hardly be imagined that, in the very short time interval involved, substantial differences in the absorbed fraction would be found. Especially since the three substances concerned have exactly the same overall physicochemical properties, are dispensed in the same formulation and to the same volunteers on an empty stomach. Accepting the significance of the differences measured it remains difficult to offer a straightforward explanation. Optical isomers can only be expected to behave differently from each other as long as three-points-interactions are involved (cf. Rodrigues de Miranda, 1975), but certainly not when general physicochemical characteristics are concerned, like acid or base strength, lipophilicity etc. Now the volume of distribution of a drug is regarded as primarily determined by its \( pK_a \), its lipophilicity and by possible affinities for cell or tissue compartments. As argued before (chapter 5) it is very unlikely that ibuprofen would cumulate in one or another tissue to a substantial amount, since most of the drug is present in plasma. A factor which definitely will play a role in the smallness of the volume is the high degree of protein binding. Small differences in affinity between the stereoisomers might not easily be detected but nevertheless be sufficient for explaining the differences in apparent volume of distribution (and to some extent also in total body clearance, cf. Van Ginneken and van Rossum, 1975). If stereoselectivity in the protein binding of ibuprofen would occur one should expect the racemate to behave as a mixture of two compounds. This is not found experimentally (Mills et al., 1973). Furthermore, Perrin (1973) demonstrated that the binding of fenoprofen, a drug closely related to ibuprofen, to human serum albumin is not stereospecific, but is mainly the result of hydrophobic interactions and hydrogen bonding of the carbonyl group. In a study on the pharmacokinetics of hexobarbital isomers Breimer (1974) also did not find significantly different protein binding percentages for the optical isomers. In general, binding to plasma proteins seems to be not stereospecific and extrapolating we assume that here no explanation for the different kinetic
behaviour of the ibuprofen isomers can be found.

On the other hand enzymatic transformation as a rule is stereoselective, so the differences found in the clearance constant are not unexpected. Then another phenomenon might be relevant for discussion, viz. a first pass effect, leading to different amounts of drug that reach the general circulation intact after oral administration. This possibility can be eliminated because of convincing arguments. For, the magnitude of the first-pass effect depends upon the ratio of hepatic drug clearance and blood (or plasma) flow through the liver. Since for the ibuprofen isomers the clearance constants are in the order of 30—50 ml/min, which is very small with regard to hepatic plasma flow (about 750 ml/min) one can conclude that none of the isomers will be cleared substantially during its first pass through the liver.

Another point of interest is the amount of ibuprofen excreted by the kidney after ingestion of any of the isomers. From the data in table 6.2 it becomes evident that ibuprofen excretion after the dextro-isomer is somewhat larger than after the levo-isomer. This is in agreement with the observation of Van Giessen and Kaiser (1975) that after administration of racemic ibuprofen most of the ibuprofen excreted is dextrorotatory. The excretion of ibuprofen-glucoronide is somewhat higher after the dextro-

TABLE 6.2

Cumulative renal excretion of ibuprofen and ibuprofen-glucuronide after oral administration of ibuprofen isomers (Q_r% = percentage of dose).

<table>
<thead>
<tr>
<th>Subject</th>
<th>isomer</th>
<th>Q_r% (unch.)</th>
<th>Q_r% (gluc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JE</td>
<td>+</td>
<td>1.42</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>0.88</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>1.33</td>
<td>8.1</td>
</tr>
<tr>
<td>LH</td>
<td>+</td>
<td>2.12</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>1.34</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>3.30</td>
<td>10.1</td>
</tr>
<tr>
<td>VR</td>
<td>+</td>
<td>3.11</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>1.04</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>3.65</td>
<td>14.3</td>
</tr>
<tr>
<td>WS</td>
<td>+</td>
<td>4.28</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>1.87</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>2.37</td>
<td>13.2</td>
</tr>
</tbody>
</table>
isomer than the levo-isomer. The observed differences are, however, only of minor importance and can in no way account for the overall pharmacokinetic differences encountered.

In this discussion up to now we neglected one additional phenomenon, which, as it will turn out, can yield the clue of the solution of our problem, viz. the possibility of isomerization. Adams et al. (1967) found that the urinary metabolites of racemic ibuprofen in man were dextro-rotatory and Mills et al. (1973) confirmed this finding, even after administration of levo-ibuprofen. A third important observation in this respect was done by Van Giessen and Kaiser (1975) who measured plasma levels of both isomers of ibuprofen separately after administration of the racemic mixture in man. The levo-enantiomer appeared to disappear from plasma much more rapidly than the dextro-enantiomer. Interestingly this seems contradictory to our data, which indicate a slightly faster elimination of ibuprofen after ingestion of the dextro- than after the levo-isomer. In combination with the differences in distribution volume we measured, this evidently suggests that stereoselective elimination alone can never satisfactorily explain the observations. On the other hand all data are in entire agreement when isomeric inversion is assumed to take place. Based upon the analysis of the simplest model including isomeric inversion as given in the appendix some numerical examples are simulated in figure 6.2. Concentration data at hourly intervals are generated for total ibuprofen after i.v. injection of the dextro- and levo-isomer and of the racemic mixture. Although the theoretical curves are principally biexponential, so that no straight lines can be expected in a semilogarithmic plot, it will in an experimental situation with a limited number of points always be possible to obtain a reasonably fitting straight line. Such lines are drawn in the figure. It is obvious that differences in the intercepts on the concentration axis exist, although the same distribution volume for both isomers was taken for simulation. This implies that also after oral administration different apparent volumes of distribution will be estimated for different isomers and that the apparent volume of distribution of the racemic mixture, which has an intermediate value, can be expected to approximate the actual volume quite well in most cases. Furthermore, it becomes clear from these simulations that the above mentioned paradox concerning the elimination rate of the two isomers can easily be accounted for. It is sufficient to assume that the high rate of disappearance, that Van Giessen and Kaiser (1975) found for the levo-isomer is primarily determined by inversion to the dextro-isomer and not at all or only for a small part by real elimination. When this inversion clearance is higher than the elimination
Figure 6.2
Theoretical plasma curves after intravenous administration of 400 mg of the separate isomers or the racemate of a hypothetical drug. The volume of distribution is 6 l for both isomers. The curves are simulated on basis of the equations given in the appendix and represent the total concentration of the drug (both isomers). The upper, middle and lower curve correspond to administration of isomer 2, racemate and isomer 1 respectively. The parameters used for simulation are: $k_{C1} = 2$, $k_{C2} = 0.2$, $k_{12} = k_{21} = 1.5$ (curve a); $k_{C1} = 2$, $k_{C2} = 1.5$, $k_{12} = 1$, $k_{21} = 0.5$ (curve b); $k_{C1} = 2$, $k_{C2} = 0.2$, $k_{12} = 0$, $k_{21} = 3$ (curve c); $k_{C1} = 2$, $k_{C2} = 1.5$, $k_{12} = 0$, $k_{21} = 3$ (curve d). Every $k$ has the dimension of $1/hr$.

It should be noted that the isomeric conversion leads to differences in the apparent volumes of distribution and that the elimination of the total amount of drug may proceed more slowly after administration of the isomer with the highest sum of isomerization and elimination clearance (curves c and d).
clearance of the dextro-isomer itself, then the elimination of total ibuprofen will be faster after the dextro-isomer than after the levo-isomer.

In conclusion we may state that our data strongly support the idea, already expressed in the literature, that the elimination of ibuprofen enantiomers involves inversion of levo- to dextro-ibuprofen, whereas the opposite inversion does not take place, or at most to a very minor extent.

NOTE added in proof:
After completion of this chapter further evidence in favour of the isomeric inversion as discussed in the text was given by Adams et al. (J. Pharm. Pharmacol. 28, 256, 1976) on basis of the difference between the pharmacological activities in vitro and in vivo and by Kaiser et al. (J. Pharm. Sci. 65, 269, 1976) on basis of measurement of the enantiomeric composition of drug-related materials excreted in human urine.

APPENDIX

The most simple pharmacokinetic model allowing interconversion of two stereo-isomers can be depicted as follows:

\[
\begin{array}{ccc}
& I_1 & \\
k_{12} & & k_{Cel_1} \\
& I_2 & \\
k_{21} & & k_{Cel_2}
\end{array}
\]

where \( I_1 \) and \( I_2 \) designate the respective isomers, \( k_{12} \) and \( k_{21} \) the 'isomerization clearance constant' (with the dimension of volume per time) and \( k_{Cel_1} \) and \( k_{Cel_2} \) the elimination clearance constant (also in volume per time). We assume the volume of distribution of both isomers to be the same. For the sake of simplicity we restrict this description to i.v. administration and then the model is governed by the following set of two simultaneous differential equations (where \( C_1 \) and \( C_2 \) represent the concentrations of the respective isomers in this single compartment, the volume of which is given by \( V \)):

\[
\frac{dC_1}{dt} = \frac{k_{21}}{V} C_2 - \frac{k_{Cel_1} + k_{12}}{V} C_1
\]
\[
\frac{dC_2}{dt} = \frac{k_{12}}{V}C_1 - \frac{k_{Cel_2} + k_{21}}{V}C_2
\] (6.3)

The boundary conditions for these equations are dependent upon the isomer administered:

for \( I_1 \) : \((t=0; C_1 = \frac{D}{V} \text{ and } C_2 = 0)\)

\( I_2 \) : \((t=0; C_1 = 0 \text{ and } C_2 = \frac{D}{V})\)

equimolar mixture of \( I_1 \) and \( I_2 \) (racemate):

\((t=0; C_1 = C_2 = \frac{D}{2V})\)

In this way the system is fully defined and both concentrations can be expressed as a function of time by integrating equation 6.2 and 6.3.

Since in the context of our discussion only the sum of concentrations of the two isomers is relevant we here only present the result for this sum \( C_1 + C_2 \). Then we obtain:

1. after i.v. administration of \( I_1 \)

\[
C = C_1 + C_2 = \frac{D}{V} \left[ \frac{k_{Cel_2} + k_{12} + k_{21}}{V} - \frac{a}{b-a} \right] e^{-at} - \frac{D}{V} \left[ \frac{k_{Cel_2} + k_{12} + k_{21}}{V} - \frac{b}{b-a} \right] e^{-bt}
\] (6.4)

2. after i.v. administration of \( I_2 \)

\[
C = C_1 + C_2 = \frac{D}{V} \left[ \frac{k_{Cel_1} + k_{12} + k_{21}}{V} - \frac{a}{b-a} \right] e^{-at} - \frac{D}{V} \left[ \frac{k_{Cel_1} + k_{12} + k_{21}}{V} - \frac{b}{b-a} \right] e^{-bt}
\] (6.5)
3. after i.v. administration of the racemic mixture \((I_1 + I_2)\)

\[
C = C_1 + C_2 = \frac{D}{V} \left[ \frac{k_{C_1} + k_{C_2} + 2k_{12} + 2k_{21}}{2V} - a \right] e^{-at} - \frac{D}{V} \left[ \frac{k_{C_1} + k_{C_2} + 2k_{12} + 2k_{21}}{2V} - b \right] e^{-bt} \quad (6.6)
\]

The rate constants (reciprocal time constants) in these equations are defined by:

\[
a, b = \frac{1}{2} (p \pm \sqrt{p^2 - 4q}) \quad (6.7)
\]

where

\[
p = \frac{k_{C_1} + k_{C_2} + k_{12} + k_{21}}{V} \quad (6.8)
\]

\[
q = \frac{k_{C_1} k_{C_2} + k_{C_1} k_{21} + k_{C_2} k_{12}}{V} \quad (6.9)
\]

REFERENCES

CHAPTER 7 COMPARISON OF THE PHARMACOKINETICS OF IBUPROFEN AND IBUFENAC IN MAN

INTRODUCTION

Among the enormous series of phenylacetic acid derivatives, synthetized and tested for antiinflammatory properties during the last 15 years, there are two compounds which interested us especially from a pharmacokinetic point of view, viz. ibufenac (4-isobutylphenylacetic acid) and its alpha-methyl analog ibuprofen (2(4-isobutylphenyl) propionic acid). Both these compounds exhibit substantial antiinflammatory, analgesic and antipyretic activity, ibuprofen being more active than ibufenac in a set of conventional animal models (ibufenac: Adams et al., 1963; ibuprofen: Adams et al., 1967). Since long term use of ibufenac occasionally caused jaundice (Buckler and Adams, 1968) the compound was abandoned and all the attention was directed towards its alpha-methyl derivative ibuprofen, which apparently lacked this side-effect. It is interesting to note that the higher incidence of side-effects for ibufenac, as compared to ibuprofen, apparently can be related to differences in tissue distribution. Studies with C\textsuperscript{14}-labelled drugs in rat and dog revealed much higher concentrations for ibufenac than for ibuprofen in liver, kidney, adrenal, fat and several other tissues after chronic administration in the same dosage during 2—4 weeks (Adams et al., 1970). However, taking into account the higher plasma concentration of ibufenac at the moment of examination, it seems that the data hardly reveal significant differences in tissue/plasma ratio's between ibufenac and ibuprofen, that have to be explained by preferential uptake of ibufenac. Although it is virtually impossible to determine tissue concentrations in man we judged it worthwhile to undertake a comparative study of the pharmacokinetics of ibuprofen and ibufenac in order to see if differences in volume of distribution and elimination rate could contribute to the differences in toxicity.

MATERIALS AND METHODS

The drugs and reagents were the same as described in chapters 2 and 5, except for the following addition: ibufenac was obtained from Boots Pure Drug Company (Nottingham, England), for which we express our gratitude.

Preparation of samples, gaschromatography and glucuronidase treatment of urine samples for both ibuprofen and ibufenac were identical to the
methods described in chapter 2. Under the circumstances described ibuprofen and ibufenac have exactly the same retention time, so that they cannot be measured separately when present in the same sample.

Drug administration
Eight volunteers participated in this study. All of them were young and healthy males, who did not receive any other medication during and for a period of at least two weeks prior to the experiments. They were fasting overnight prior to drug administration and tor at least 2.5 hrs thereafter. Every volunteer received both ibuprofen and ibufenac at a biweekly interval. Both drugs were administered in a dose of 400 mg in the form of soft gelatine capsules, containing 200 mg of pure drug substance. They were taken together with about 200 ml of water.

RESULTS AND DISCUSSION

The pharmacokinetic parameters obtained are summarized in table 7.1. An illustrative example of the plasma and renal excretion data is given in fig. 7.1. All parameters are based upon computerized analysis according to the open one-compartment model which yields an adequate description of the experimental findings. In the following these parameters are discussed in terms of the elementary processes involved.

Absorption
As a rule ibufenac appears to be absorbed much more slowly than ibuprofen. Ibufenac absorption is also more irregular. The reason of this observation is not self-evident, although differences in lipophilicity may have something to do with it. As shown in chapter 3 ibuprofen is more lipophilic than ibufenac, which might give rise to a faster penetration through the mucosa of the gastrointestinal tract. The somewhat lower acidity of ibuprofen ($pK_a = 4.4$) as compared to ibufenac ($pK_a = 4.1$; Skidmore and Whitehouse, 1967) might enhance the permeation difference, although only to a minor extent. According to generally accepted theories gastrointestinal absorption of organic acids is increasing with increasing lipophilicity (provided that low watersolubility does not become a limiting factor) and with increasing $pK_a$, since only the unionized acid readily crosses the membraneous barriers of the gastrointestinal tract (Schanker et al., 1957, 1958, 1959; Hogben et al., 1957, 1959). It is questionable, however, whether the relatively small differences in physicochemical properties of the two drugs under consideration can be solely
TABLE 7.1

Comparative pharmacokinetic parameters for ibuprofen and ibufenac after oral administration (D=400 mg; capsule).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>V/F (1)</th>
<th>k_{Cel}/F (ml/min)</th>
<th>( \tau_{el} ) (min)</th>
<th>( t^{\frac{1}{2}} ) (min)</th>
<th>( \tau_{a} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JA (22,70,181)</td>
<td>ibuprofen</td>
<td>7.6</td>
<td>49</td>
<td>156</td>
<td>108</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>11.5</td>
<td>54</td>
<td>213</td>
<td>148</td>
<td>183</td>
</tr>
<tr>
<td>DB (20,80,185)</td>
<td>ibuprofen</td>
<td>8.6</td>
<td>53</td>
<td>160</td>
<td>111</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>22.7</td>
<td>110</td>
<td>210</td>
<td>146</td>
<td>90</td>
</tr>
<tr>
<td>GB (20,65,178)</td>
<td>ibuprofen</td>
<td>9.2</td>
<td>54</td>
<td>170</td>
<td>118</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>12.8</td>
<td>85</td>
<td>151</td>
<td>104</td>
<td>24</td>
</tr>
<tr>
<td>BK (25,60,173)</td>
<td>ibuprofen</td>
<td>5.7</td>
<td>38</td>
<td>153</td>
<td>106</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>12.3</td>
<td>68</td>
<td>182</td>
<td>126</td>
<td>88</td>
</tr>
<tr>
<td>HL (23,76,183)</td>
<td>ibuprofen</td>
<td>6.4</td>
<td>36</td>
<td>178</td>
<td>124</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>10.7</td>
<td>36</td>
<td>299</td>
<td>207</td>
<td>31</td>
</tr>
<tr>
<td>TS (20,67,180)</td>
<td>ibuprofen</td>
<td>8.3</td>
<td>53</td>
<td>155</td>
<td>108</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>12.8</td>
<td>130</td>
<td>102</td>
<td>71</td>
<td>102</td>
</tr>
</tbody>
</table>

1. Numbers in parentheses represent age (yr), bodyweight (kg) and height (cm); all subjects are male.
Figure 7.1
Plasma curves and renal excretion of ibuprofen and ibufenac after oral administration (400 mg, capsule) to the same volunteer.
responsible for the different absorption characteristics. A contributing factor might also be the binding to plasma proteins. It is conceivable that by strong protein binding in plasma the concentration gradient of free, diffusible drug from the lumen towards the vascular system surrounding the gastrointestinal tract is kept high. This phenomenon obviously can enhance the absorption process, which is assumed to be governed by passive diffusion. Now ibuprofen is known to be almost completely bound to plasma proteins at usual concentrations whereas exact data are unfortunately not available for ibufenac. However, on the basis of the lower lipid solubility of ibufenac it seems possible that also its protein binding will be lower than that of ibuprofen.

With regard to the mechanism of the absorption one last remark should be made. An increasing number of reports is recently appearing in literature which suggest that the common theories on passive diffusion as the main determinant of the absorption process, are open to question again. For several substances it is known that active processes are involved in absorption (Levy and Jusko, 1966; Rivier, 1973; Giorgi, 1970; Gibaldi and Grundhofer, 1972; Lauterbach, 1975; Csáký, 1975).

If this would be the case also for ibuprofen and ibufenac, then the differences in absorption rate could be primarily attributed to differences in the affinity for and the capacity of the carrier mechanism involved. But as long as no direct evidence in favour of a carrier mediated absorption is provided, these considerations remain purely speculative.

**Volume of distribution**

Since the drugs in this study are administered orally the distribution volume cannot be determined in absolute terms, but only relatively to the biological availability. Table 7.1 shows the best possible estimates for the ratio V/F for both ibuprofen and ibufenac. The data strongly indicate a larger apparent volume of distribution for ibufenac than for ibuprofen. This is in accordance with the studies of Adams et al. (1970) who found that ibufenac in animals reached much higher tissue levels than ibuprofen. On the other hand it is contrary to what might be expected on the basis of lipid solubility. Again (as in the discussion above) differences in protein binding might be the crucial determinant.

Some variation in the estimates of V/F will also be caused by differences in biological availability. The more irregular and less rapid absorption of ibufenac as compared to ibuprofen might result in a relatively smaller amount absorbed.

However, as the data in table 7.1 show, there is no correlation between the
time constant for absorption and the estimated value for \( V/F \) after ibufenac administration.

Accepting the supposition that ibufenac actually has a larger volume of distribution than ibuprofen, it remains questionable whether this is significant in the sense that it might be related to different tissue distribution. Although there are some indications for higher tissue levels of ibufenac than of ibuprofen, we therefore tend to conclude that the observed toxicity of ibufenac is a specific effect, which is not or only to a minor extent exhibited by ibuprofen.

**Elimination**

Since the predominant symptom of toxicity of ibufenac was the occurrence of jaundice, one might speculate that ibufenac affects the metabolic system in the liver. Then also ibutenac itself might be eliminated at a slow rate as compared to ibuprofen, or at least exhibit an irregular elimination pattern. However, although the rate of elimination of ibufenac in most cases indeed was found to be lower than that of ibuprofen, in some of the volunteers the opposite was true. The best measure for the efficiency of the clearing organs with respect to a certain drug is not the time constant or the rate constant for elimination, but rather the clearance constant. Since the clearance constant for ibufenac tends to be even higher than that of ibuprofen (although we only can compare the relative values \( k_{\text{Ce1}}/F \) and since the clearance is practically confined to hepatic metabolism, there is no indication at all for impaired hepatic function with respect to ibufenac. The presupposition of irregular elimination was confirmed by preliminary experiments with ibufenac. Already in our ibuprofen studies we noticed that in a few cases a small oscillation occurred in the elimination phase. This oscillatory behaviour was interesting to note, but not of practical importance, since the amplitude of the oscillation was low and in no way a hindrance to optimal determination of the slope of the semi-logarithmic plasma decay curve. This situation altered in the first ibufenac experiments, where the incidental oscillations took the form of real drops in the plasma curve (cf. figure 7.2). It became evident that a drop in the plasma curve (indicating a temporarily enhanced elimination) only occurred after the volunteers involved had taken a quite heavy dinner. When only a light lunch was taken no significant oscillation was noticed. It is an attractive hypothesis therefore to attribute these phenomena to an enterohepatic circulation of the drug or at least to an enhanced biliary excretion. For, it is conceivable that increase in bile secretion (under the influence of fatty food components) promotes biliary excretion of the
Figure 7.2
Plasma curve as obtained after oral administration of ibufenac in a pilot experiment. Note the drop in the curve, which coincides with dinner. See text for further explanation.

drug or its metabolites (e.g. glucuronide), which afterwards may be re-absorbed from the intestine. When such an effect becomes very pronounced, it should be realized that the area under the plasma curve, which normally is equal to $\frac{FD}{k_{Cel}}$, will be seriously overestimated. An entero-hepatic circulation of ibuprofen has been suggested in animal experiments by Mills et al (1973). We obtained evidence that in man the phenomenon is much more significant for ibufenac. This implies that, depending upon the composition of the diet, the elimination of ibufenac may be seriously retarded and in consequence toxicity eventually increased.

Renal excretion
As a rule unchanged ibufenac is excreted in a lower percentage than ibuprofen. Since only very minor amounts appear in urine unchanged this observation is only of academic interest, without practical consequences.
TABLE 7.2

Cumulative renal excretion of unchanged and glucuronidated drug after oral administration of ibuprofen and ibufenac (D=400 mg; capsule) (\(Q_r\% = \% \text{ of dose}\)).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>(Q_r%) (unch.)</th>
<th>(Q_r%) (gluc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JA</td>
<td>ibuprofen</td>
<td>0.83</td>
<td>13.90</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>0.88</td>
<td>1.00</td>
</tr>
<tr>
<td>DB</td>
<td>ibuprofen</td>
<td>1.45</td>
<td>8.85</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>0.75</td>
<td>6.25</td>
</tr>
<tr>
<td>GB</td>
<td>ibuprofen</td>
<td>0.35</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>0.45</td>
<td>3.55</td>
</tr>
<tr>
<td>BK</td>
<td>ibuprofen</td>
<td>0.80</td>
<td>9.25</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>0.23</td>
<td>4.80</td>
</tr>
<tr>
<td>HL</td>
<td>ibuprofen</td>
<td>2.06</td>
<td>16.20</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>0.55</td>
<td>2.70</td>
</tr>
<tr>
<td>TS</td>
<td>ibuprofen</td>
<td>0.43</td>
<td>10.90</td>
</tr>
<tr>
<td></td>
<td>ibufenac</td>
<td>0.28</td>
<td>4.75</td>
</tr>
</tbody>
</table>

It was intriguing to find that, contrary to ibuprofen, ibufenac did not appear to be subject to tubular secretion mechanisms. Actually an opposite behaviour was found, for the renal clearance constant of ibufenac tended to increase with increasing plasma concentration. A typical example is given in figure 7.3, where for the same volunteer the renal excretion rate is plotted as a function of the average plasma concentration. Ibuprofen exhibits the usual pattern of combined filtration and secretion (cf. chapter 5) but ibufenac excretion seems to be inhibited at low plasma concentration. One way of explaining this phenomenon is to assume that ibufenac is actively reabsorbed from tubular urine, which can result in a clearance lower than the degree of filtration. Perhaps more likely ibufenac’s excretion in urine is limited by binding to plasma proteins. It seems hardly plausible that the rather non-specific mechanism, that accomplishes tubular secretion of ibuprofen, would not affect ibufenac. On the other hand it is quite conceivable that the mechanism would be less efficient with respect to ibufenac. In that case it is possible that the protein binding conceals all signs of the secretion process and that a curve as depicted in figure 7.3 results (Rodrigues de Miranda, 1975).
Figure 7.3
Renal excretion rate as a function of the average plasma concentration over the excretion interval for ibuprofen and ibufenac in the same volunteer. Whereas ibuprofen shows clear signs of a tubular secretion, the opposite is true for ibufenac. See text for further explanation.

Turning to the data on renal excretion of the glucuronides, we observe that after equal doses less ibufenac than ibuprofen is excreted in urine in the form of its glucuronide. It is, however, an unallowed extrapolation to consider the amount of glucuronide in the urine as a reflection of the capacity of the organism to perform the conjugation reaction. Since biliary excretion will be an important pathway of elimination at least of ibufenac and probably also for ibuprofen, one must assume that major fractions of glucuronide are excreted in bile. After splitting by the intestinal flora part of the drug may be reabsorbed (enterohepatic circulation). So, obviously a difference in the rate of renal excretion of the glucuronide should not be ascribed a priori to a difference in the rate of glucuronide formation.
REFERENCES

CHAPTER 8 PHARMACOKINETICS OF ALCLOFENAC IN MAN

INTRODUCTION

Among the series of arylacetic acid derivatives, used in the therapy of inflammatory disorders, alclofenac* (4-allyloxy-3-chlorophenylacetic acid) attracts the attention because it combines the usual antiinflammatory and antipyretic effectiveness with a more general analgetic action. In this sense the pattern of pharmacological activity seems to be comparable to that of acetylsalicylic acid. In a clinical double-blind study alclofenac (500 mg) was more effective than codeine (30 mg) and about as potent as pentazocine (50 mg) in the treatment of chronic pain (Staquet et al., 1971). The analgesic effect appears to be directly related to the plasma levels (Roncucci et al., 1971). Lambelin et al. (1970) reported that in hyperthermic rabbits alclofenac is 45 times more active than acetylsalicylic acid. Although in this respect no systematic clinical studies have been done up to now alclofenac can be expected to be of value as antipyretic agent. As far as the antiinflammatory effect is concerned alclofenac has been compared to various other drugs in the treatment of rheumatic diseases (Fellmann, 1971; van Hoek, 1970; Aylward, 1973, 1975; Pavelka et al., 1973; Klemm et al., 1971). Alclofenac was found equipotent or sometimes superior to the other drugs studied (ibuprofen, indomethacin, phenylbutazone) and often better tolerated. Apart from slight gastrointestinal complaints and incidental allergic skin reaction no significant side-effects were reported. Billings et al. (1974), however, reported three cases of a hypersensitivity vasculitis which disappeared after withdrawal of alclofenac.

Extensive studies on the metabolic pattern of alclofenac in several animal species, including man, have been reported (Roncucci et al., 1970, 1971, 1972). Appreciable interspecies differences were noted but in all cases practically the whole dose absorbed was excreted in urine, partly as unchanged alclofenac, partly as metabolites. In man about 70% of the dose was excreted in urine as alclofenac or alclofenac glucuronide and another 20% in the form of two other metabolites (3-chloro-4-hydroxyphenylacetic acid and 3-chloro-4-dihydroxypropyloxyphenylacetic acid). Also some data on the pharmacokinetics of radiolabelled alclofenac in patients with various disorders were published (Roncucci et al., 1971), as well as a most interesting discussion on dosage regimens for chronic therapy with

* Mirvan®, reg. trademark Continental Pharma, Brussels, Belgium.
alclofenac (Strolin-Benedetti et al., 1973).

The present investigations were undertaken in connexion with a comparative study on the pharmacokinetics of various antiinflammatory analgesics. Our purpose was to look after relationships of kinetic behaviour with physicochemical characteristics and to collect data for planning rational dosage regimens.

MATERIALS AND METHODS

Drugs

Alclofenac pure substance for reference purposes was obtained from Continental Pharma, Brussels, Belgium, which is gratefully acknowledged. Mirvan® tablets and suppositories (containing 500 resp. 600 mg of alclofenac) were commercially obtained as they are marketed in the Netherlands.

Preparation of samples and gaschromatographic analysis

As described in chapter 2.

Drug administration

The ten volunteers participating in this study were all young, healthy males, who did not receive any other medication during and for a period of at least 4 weeks prior to the experiments. None of them has ever suffered from liver or kidney function impairment. They were fasting overnight prior to and for at least 3 hrs after drug intake (at nine o’clock in the morning). All volunteers received an oral dose of 500 mg alclofenac in the form of commercially available tablets (Mirvan®). Four of them also took a rectal dose of 600 mg alclofenac in the form of a Mirvan® suppository. Two of the volunteers ingested an oral dose of 1000 mg (two tablets). Oral doses were always taken together with about 100 ml of water. In all single dose experiments about 10 blood samples were taken from the forearm vein at regular times after drug intake and all urine samples were collected separately for a period of at least 30 hrs. One volunteer took alclofenac chronically for two weeks in a dosage regimen of one tablet three times daily (at 1.00, 9.00 and 17.00 hrs). Blood samples were collected frequently during the first dosage interval and after the last dose and an additional two or three every other day over the whole period of drug administration.
RESULTS AND DISCUSSION

Some representative examples of the plasma levels and urinary excretion of unchanged drug after oral and rectal administration of alclofenac are given in figures 8.1, 8.2 and 8.3. Table 8.1 summarizes the relevant pharmacokinetic parameters as obtained by computerfit (non-linear regression program Farmfit) according to the open one compartment model (see chapter 4). Already on visual inspection of the experimental data it was obvious that this pharmacokinetic model would provide a very satisfactory description of alclofenac pharmacokinetics. This was evidently confirmed by the computer analysis as can be seen from the figures. The drawn lines actually are the curves obtained by the computerized regression analysis and they match the measured points excellently.

**TABLE 8.1**

Pharmacokinetic parameters after oral and rectal administration of alclofenac.

<table>
<thead>
<tr>
<th>Subject</th>
<th>V/F (1)</th>
<th>k\text{Ce}/F (ml/min)</th>
<th>\text{t}_1\text{/2} (min)</th>
<th>\text{t}_a (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>6.2</td>
<td>37</td>
<td>166</td>
<td>115</td>
</tr>
<tr>
<td>AA³</td>
<td>9.0</td>
<td>53</td>
<td>170</td>
<td>118</td>
</tr>
<tr>
<td>JB</td>
<td>5.6</td>
<td>38</td>
<td>147</td>
<td>102</td>
</tr>
<tr>
<td>MB</td>
<td>8.9</td>
<td>38</td>
<td>235</td>
<td>163</td>
</tr>
<tr>
<td>JE</td>
<td>7.5</td>
<td>44</td>
<td>171</td>
<td>119</td>
</tr>
<tr>
<td>JH</td>
<td>4.3</td>
<td>41</td>
<td>104</td>
<td>72</td>
</tr>
<tr>
<td>LH</td>
<td>5.1</td>
<td>45</td>
<td>114</td>
<td>79</td>
</tr>
<tr>
<td>PK</td>
<td>7.1</td>
<td>49</td>
<td>145</td>
<td>101</td>
</tr>
<tr>
<td>WM</td>
<td>6.1</td>
<td>40</td>
<td>152</td>
<td>106</td>
</tr>
<tr>
<td>DZ</td>
<td>10.0</td>
<td>69</td>
<td>146</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>ORAL, 500 mg (tablet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>16.2</td>
<td>70</td>
<td>231</td>
<td>160</td>
</tr>
<tr>
<td>PK</td>
<td>16.7</td>
<td>107</td>
<td>156</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>ORAL, 1000 mg (2 tablets)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>9.5</td>
<td>67</td>
<td>143</td>
<td>100</td>
</tr>
<tr>
<td>JB</td>
<td>9.5</td>
<td>47</td>
<td>202</td>
<td>140</td>
</tr>
<tr>
<td>WM</td>
<td>7.8</td>
<td>48</td>
<td>163</td>
<td>113</td>
</tr>
<tr>
<td>DZ</td>
<td>9.6</td>
<td>68</td>
<td>141</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>RECTAL, 600 mg (supp.)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Numbers in parentheses represent age (yr), bodyweight (kg) and height (cm) respectively; all subjects are male.
2. Parameters determined after chronic administration (500 mg 3 dd) for 2 weeks.
Figure 8.2
Plasma curves and cumulative renal excretion after administration of alclofenac in doses of 500 and 1000 mg to the same subject. See text for further explanation.

Figure 8.1
Plasma curves of alclofenac after oral and rectal administration to the same subject.
Absorption rate
When administered on an empty stomach the absorption of alclofenac tablets is a fairly rapid process. The time constant for absorption, based upon the assumption of a unidirectional first-order process, usually does not exceed 40 minutes and is much shorter in several cases. This indicates that the maximum plasma level will be obtained within about 2 hrs after oral administration of the tablets to fasting subjects. In our study the average time constant for absorption is definitely smaller than the average value of 110 minutes found by Roncucci et al. (1971) (cf. Strolin-Benedetti et al., 1973). Two factors may be primarily responsible: firstly, they employed a different drug formulation (soft gelatine capsules) and secondly, their subjects were non-fasting patients. Since from our preliminary investigations we got the impression that the absorption was not retarded at all by a light breakfast, we tend to believe that here a real biopharmaceutical difference is concerned and that alclofenac absorption from commercial tablets occurs more rapidly than from gelatin capsules. The fact that two of our volunteers show an unusually slow absorption, most probably has to be attributed to unknown, individual factors not representing a drug- or formulation-characteristic. This is the more so since one of them (JB) after rectal administration absorbed the drug at a normal high rate.

Also from suppositories alclofenac is quite rapidly absorbed with a rate in the same range as found for the tablets.

With regard to the onset of the absorption process our data indicate no appreciable delay after oral or rectal administration. The regression analysis did not improve when the possibility of a lag-time was incorporated. Therefore we conclude that absorption starts immediately after administration. This conclusion should, however, be restricted to our experimental setting where the drug is administered to fasting volunteers. It is quite possible that the presence of a substantial amount of food in the gastrointestinal tract will give rise to a limiting step before normal first order absorption can occur (cf. also chapter 4). On the other hand, as stated above, the final rate of absorption does not seem to be very sensitive to such factors. With respect to the rapid onset of absorption an exception has to be mentioned, viz. the 1000 mg doses in the two subjects MB and PK where optimal fit required a lag time of 43 and 31 min respectively. Although this observation may be reminiscent of dose dependent pharmacokinetics, we have no evidence that such dose dependence actually exists. On the contrary, the profile of the plasma curve after the high dose fits well into the normal one compartment model with
first-order absorption and elimination. Moreover, data by Roncucci et al. (1971) are in flat contradiction with non-linear kinetics. Therefore our observation may be a mere coincidence that should be investigated in more detail before definite statements can be made. There are, however, still other remarkable aspects involved in this matter as will be discussed below.

**Elimination rate**

Alclofenac is rapidly eliminated from the body. The halflives we measured varied roughly from 1.5 to 2.5 hrs. The rate of elimination is independent of the dose and the pharmaceutical formulation of the drug, in so far as in our study no significant differences were found in this respect. The short halflife implies that it will be very difficult to obtain a constant plateau concentration of alclofenac, which might be desirable for optimal therapy in chronic inflammatory disorders. On the other hand, it diminishes the risk of accumulation to toxic levels. This can certainly be of advantage when alclofenac is used as a general analgesic agent, since then the drug can be taken frequently for a short period of time. Of course, one has to take into account that the range of halflives of alclofenac for the whole population will be wider than what we found in our limited and homogeneous group of volunteers. An indication in favour of this expectation can be found in the literature (Strolin-Benedetti et al., 1973, reporting values of 2.5 to 5.5 hrs in an inhomogeneous group of 5 elderly patients).

Assuming that effective analgesia with alclofenac requires plasma levels of about 10 mg/l (Roncucci et al., 1971) one may expect that in our study therapeutic blood levels lasted for 3–5 hrs, starting within 20 min after oral or rectal administration. Antiinflammatory effects of course cannot easily be quantified, improvement of clinical symptoms should guide long term therapy.

**Volume of distribution and biological availability**

Since we are dealing with oral and rectal administration our analysis does not yield exact values of the volume of distribution (and of the clearance constant) since we cannot know a priori which fraction of the dose administered actually has been absorbed. Therefore it is inevitable to combine the discussion of the fundamental parameters (V and $k_{Ce1}$) with consideration of the biological availability (F). The total urinary and faecal excretion of radioactivity after administration of alclofenac-14C suggests that large differences in the biological availability may occur (Roncucci et al., 1971). In some cases Roncucci got practically complete recovery in urine, in some other cases large amounts appeared in the faeces. The most
logical explanation is that in the latter cases the absorption has been far from complete, since all patients had normal renal and hepatic function.

Because our estimated values for the volume of distribution in fact represent the ratio \( V/F \) we may assume that at least part of the variations in these estimates results from differences in the fraction absorbed (F). This implies that the tabulated individual values actually represent the upper limit for the volume of distribution of alclofenac in a one-compartment model. Obviously then the volume of distribution is very small (5 to 7 l, cf. also the discussion on ibuprofen in chapter 5). Most probably binding to plasma proteins is a factor contributing to the small distribution volume. Although exact data are not available, preliminary data suggest that alclofenac is extensively bound to human plasma proteins, but definitely less than for instance ibuprofen. On the other hand alclofenac is less lipophilic than ibuprofen, so that it will have more difficulties in passing through biological membranes. The combination of these factors seems to result in comparable distribution volumes for alclofenac and ibuprofen.

Assuming that the real volume of distribution is exclusively determined by the physicochemical characteristics of the drug and not by its dose and formulation, we can estimate the relative biological availability of different drug formulations by comparing the ratios \( V/F \) calculated after administering the dosage forms. In case of alclofenac this comparison leads to the conclusion that the biological availability of the suppository in three of our volunteers is lower than that of the tablet, the ratio \( \frac{F_{\text{supp.}}}{F_{\text{tabl.}}} \) being 0.65; 0.59 and 0.78 for the subjects AA, JB and WM respectively. In one case the ratio is about unity (1.04 for subject DZ), but in this case also the oral dose seems to be only partially absorbed. So there is some indication that the bioavailability of the suppository is a little lower than that of the tablet, but the availability of the tablet is fluctuating also. Since furthermore the suppository contains a higher dose (600 mg instead of 500 mg) there will be no need at all for adapting the dosage regimen when tablets and suppositories are administered alternately. Also for single dose situations the two forms are equally effective.

A few remarks should be made concerning the 1000 mg doses, in these cases a strikingly high estimate of \( V/F \) resulted. Together with the unusual lag-time mentioned above, this may be due to an irregular absorption process. It can easily be derived that in our analysis a too high apparent value for the volume of distribution would be obtained when for instance the so-called lag-time were a period during which absorption does take
place in fact but with a rate lower than in the ultimate first-order phase. The shorter the halflife of the drug the more pronounced this phenomenon will be. Taking into account these considerations a rough estimate of the error made in our analysis indicates that it might amount to some 50% overestimation of the distribution volume. This of course would bring the values for V/F more in line with the normal range found for the 500 mg dose. In general this example points to a weak side in pharmacokinetic analysis: when the absorption process is not purely first order and unidirectional large errors may be encountered in the calculation of the volume of distribution (and the clearance constant). In such cases due reserve should be practised in the presentation of the results.

Clearance
Also the clearance constant cannot be determined in absolute terms after oral administration, only the ratio \( k_{\text{Cel}} \) relative to \( F \) can be given (table 8.1). Referring to the factors discussed above we may give for the expectation-values of the clearance constant the range \( k_{\text{Cel}} = 30-50 \text{ ml/min} \). This range is comparable to ibuprofen (chapter 5) and obviously no first-pass effect can be expected since the overall clearance constant is very small with respect to hepatic plasmaflow. Furthermore in case of alclofenac usually less than 80% of the total clearance occurs by metabolism, the rest being urinary excretion. Excretion into the faeces is not an important pathway since in man practically the whole dose appears in urine, partly in the form of metabolites (Roncucci et al., 1970, 1971). It should be noted that the clearance constant for alclofenac is quite low, indicating an inefficient extraction in the clearing organs. As was argued before for ibuprofen (chapter 5), protein binding may be a limiting factor. The hepatic clearance may also be hindered somewhat by the fact that alclofenac is not as lipophilic as a drug like ibuprofen is, so that it probably cannot penetrate into the liver cells as easily as ibuprofen. On the other hand this might be accompanied by a higher watersolubility which can be in favour of urinary excretion by glomerular filtration, because passive backdiffusion from primary urine is less likely to occur.

Renal excretion
Alclofenac is excreted in urine unchanged in very variable amounts. The variations are much larger than could be explained on basis of differences in the fraction of the dose that has been absorbed. As table 8.2 shows none of our volunteers excreted less than 10% of the dose unchanged whereas the other extreme amounts to not less than 50%. The percentages
TABLE 8.2

Cumulative renal excretion of alclofenac as % of dose ($Q_r\%$).

<table>
<thead>
<tr>
<th>Subject</th>
<th>$Q_r%$</th>
<th>Subject</th>
<th>$Q_r%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA, o</td>
<td>14</td>
<td>JH, o</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>LH, o</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JB, o</td>
<td>14</td>
<td>PK, o (500)</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>o (1000)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB, o (500)</td>
<td>36</td>
<td>WM, o</td>
<td>48</td>
</tr>
<tr>
<td>o (1000)</td>
<td>13</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JE, o</td>
<td>31</td>
<td>DZ, o</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

1. "o" and "r" refer to oral or rectal administration.

Figure 8.3
Cumulative renal excretion of alclofenac after oral or rectal administration to the same volunteer whose plasma curves are represented in figure 8.1. The renal clearance is related to the urine flow. See text for further explanation.
are in no way correlated to the estimates of V/F, so apart from the fact that they are too variable to be caused by changes in F they fluctuate fully independently of it. Since renal excretion of unchanged drug is a major pathway of elimination for alclofenac, it is worthwhile to examine the factors that may influence this excretion, in more detail. When dealing with ionizable compounds one has to consider of course the influence of urinary pH on the net excretion. As argued before (chapter 3) it is not enough to reckon with the degree of ionization of the drug in urine but also its lipophilicity plays an important role. Even drugs that are ionized in urine for more than 99% can be reabsorbed substantially when their lipophilicity is very high. Alclofenac has a pKₐ of about 4.6, which implies that at the urinary pH values we measured in our volunteers (pH ≥ 5.5) usually more than 90% of the drug in urine is ionized. Now the lipophilicity of alclofenac is certainly not high enough to compensate fully for this high degree of ionization (see chapter 3), so that we may expect that passive backdiffusion will not prevent substantial renal excretion. In man the glomerular filtration rate is about 125 ml/min. The renal clearance constant for alclofenac in our study is definitely much lower and never exceeds 35 ml/min. In other words: the renal clearance is far below the maximally attainable value, even when no tubular secretion occurs at all. In this context one should regard the binding to plasma proteins as a predominant factor and backdiffusion as a cooperative mechanism. As long as this backdiffusion is independent of urinary pH and only governed by the concentration of the drug in the glomerular filtrate and the resulting tubular urine, it offers no special problems from a pharmacokinetic point of view. For, in such a situation the only consequence is that filtration and backdiffusion together sum up to an effective renal clearance constant, lower than the original filtration rate, but highly significant for practical purposes. This situation seems to prevail in case of alclofenac. Although in all individuals the pH of the urine samples collected was rather variable (5.5—7.2) we were unable to detect related fluctuations in the renal excretion rate. As argued above this is not very surprising on the basis of physicochemical properties of alclofenac. On the other hand it was intriguing to note that the same individual on different occasions showed large differences in the cumulative amount of drug excreted unchanged. Interestingly this appeared to be connected with the total volume of urine produced over the excretion period. For the volunteers that participated twice in this study, table 8.3 shows the percentage of the dose excreted unchanged (Qᵣ %) together with the average urine flow (in ml/min) and the upper limit of the overall clearance constant (kₑl/F) over the total
excretion period (about 30 hrs). Since we are dealing with variable bioavailability the last column contains the value for \( k_{Cr} \), the renal clearance constant, which is independent of \( F \) because it can be calculated from the product of \( Q_r \) and \( k_{Cel}/F \), where \( Q_r \) is related to the total dose administered and as a matter of fact represents \( F.Q_r \). Obviously these data suggest a positive correlation between the amount excreted unchanged and the urine flow. It should be noted that the renal clearance constant in any case is much higher than the urine flow, although much lower than the rate of glomerular filtration (see above). This phenomenon is highly interesting since it indicates that during the passage of the glomerular filtrate from the glomerulus through the tubuli and the collecting tubes to the ureter no full equilibrium exists between the primary urine and the surrounding blood-vessels. For if this were the case the ultimate renal clearance constant should approach the urine flow rate more closely. On the other hand some reabsorption (backdiffusion) must occur since otherwise the renal clearance would be independent of the urine flow, but only dependent on the efficiency of the glomerular filtration.

### TABLE 8.3

Renal excretion data of alclofenac for volunteers who participated twice in this study.

<table>
<thead>
<tr>
<th>Subject¹</th>
<th>( Q_r % )</th>
<th>urine-flow (ml/min)</th>
<th>( k_{Cel}/F ) (ml/min)</th>
<th>( k_{Cr} ) (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA, o</td>
<td>14</td>
<td>0.60</td>
<td>37</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.91</td>
<td>53</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.56</td>
<td>67</td>
<td>8.0</td>
</tr>
<tr>
<td>JB, o</td>
<td>14</td>
<td>0.47</td>
<td>38</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.36</td>
<td>47</td>
<td>4.7</td>
</tr>
<tr>
<td>MB, o (500)</td>
<td>36</td>
<td>2.40</td>
<td>38</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.70</td>
<td>70</td>
<td>9.1</td>
</tr>
<tr>
<td>PK, o (500)</td>
<td>40</td>
<td>2.00</td>
<td>49</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.07</td>
<td>107</td>
<td>12.8</td>
</tr>
<tr>
<td>WM, o</td>
<td>48</td>
<td>2.46</td>
<td>40</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.00</td>
<td>48</td>
<td>9.6</td>
</tr>
<tr>
<td>DZ, o</td>
<td>50</td>
<td>2.77</td>
<td>69</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.07</td>
<td>68</td>
<td>13.6</td>
</tr>
</tbody>
</table>

¹ "o" and "r" refer to oral or rectal administration.
With regard to the influence of urine flow on the efficiency of the renal excretion we wondered if this might be a general phenomenon that also would be detectable when data from several subjects together were compared. Some indications that this extrapolation is allowed indeed, can be derived from figure 8.4. Taking all excretion data from our study together we plotted the percentage of drug excreted against the urine flow. The same type of graph was constructed for the urinary clearance constant. A tendency to positive correlation is certainly present and mathematical analysis yielded highly significant correlation coefficients (see legend to figure 8.4). The dependence of renal clearance of a drug on the urinary flow rate is a possible phenomenon that should be considered in pharmacokinetic studies on basis of renal excretion data. Usually not enough attention is paid to this possibility (Fülgraff, 1972).

![Figure 8.4](image)

**Figure 8.4**
Correlation between the amount of alclofenac excreted unchanged, $Q_r$ (and the renal clearance constant $k_{Cr}$) and the average urine flow over the period in which substantial excretion occurred. The drawn lines are the result of the regression analysis. Since the intercepts did not differ significantly from 0, also regression lines through the origin were calculated (dotted).
The next point of interest obviously is the question whether also a relation can be found between the urine flow (and consequently the renal excretion rate) and the total rate of elimination. Since urinary excretion can play a major role in the elimination process one might presume that factors decreasing the rate of urinary excretion generally will decrease the overall rate of elimination as well. Contrary to what we expected, this is not found at all. No straightforward relation between these rates could be demonstrated. Strangely enough, one gets the impression that low urinary excretion is effectively counterbalanced by high metabolism. This is quite an unusual observation but it is substantiated by the finding that the rate of urinary excretion of alclofenac glucuronide increases when that of free unchanged compound decreases. Table 8.4 shows the excretion percentages of both unchanged and glucuronidated alclofenac for those experiments in which we performed glucuronidase treatment of the urine samples. When one takes the variations in bioavailability into account, there appears a striking constancy of the sum of the amounts of free and glucuronidated drug excreted. For the moment it is impossible to offer a satisfactory explanation for this phenomenon. One might speculate about the possibility of conjugation in the kidney, such as described for benzoic acid, salicylic acid and p-aminobenzoic acid in animals (Suk Han Wan and Riegelman, 1972a, b; Suk Han Wan et al., 1972). Alternatively one might think of the possibility of splitting of substantial amounts of previously formed glucuronide by glucuronidases in the kidney. Our data, however,

**TABLE 8.4**

Cumulative renal excretion of unchanged and glucuronidated alclofenac after oral and rectal administration.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Q_r% (unch.)</th>
<th>Q_r% (gluc.)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA, o</td>
<td>14</td>
<td>68</td>
<td>82</td>
</tr>
<tr>
<td>r</td>
<td>12</td>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>JB, o</td>
<td>14</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>r</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>WM, o</td>
<td>48</td>
<td>14</td>
<td>62</td>
</tr>
<tr>
<td>r</td>
<td>20</td>
<td>36</td>
<td>56</td>
</tr>
<tr>
<td>DZ, o</td>
<td>50</td>
<td>18</td>
<td>68</td>
</tr>
<tr>
<td>r</td>
<td>20</td>
<td>42</td>
<td>62</td>
</tr>
</tbody>
</table>
Renal excretion rate of alclofenac as a function of the plasma concentration. A linear relationship prevails. See text for further explanation.

Up to now we implicitly assumed that the renal excretion of alclofenac is governed by linear processes. The argumentation presented might become disputable when substantial amounts of alclofenac would be excreted in urine by a tubular secretion mechanism. As was shown before (chapter 5) evidence concerning the mechanisms of renal excretion can be obtained by studying the excretion rate as a function of the average plasma concentration over the excretion interval. In case of alclofenac the renal excretion rate appears to be a linear function of the plasma concentration, indicating that only linear processes (presumably filtration and some passive backdiffusion) contribute significantly to the overall excretion (see fig. 8.5). This means that the assumption expressed above for all practical purposes is justified. However, it should be realized that these data do not fully exclude the presence of a tubular secretion mechanism. The renal excretion rate in case of combined filtration and secretion can be represented by

\[
\frac{dQ_r}{dt} = \left(k_{Cr} + \frac{T_M}{K_T + C_p}\right) C_p
\]  

(8.1)
Explanation of the symbols is given already in chapter 5. This equation shows that as long as \( C_p \ll K_T \) a purely linear function results, viz.

\[
\frac{dQ_r}{dt} = \left( k_{Cr} + \frac{T_M}{K_T} \right) C_p
\]  

(8.2)

Of course this rate is experimentally indiscernable from filtration alone. In view of the wide range of plasma concentrations involved, this situation would only prevail when the drug would have an extremely low affinity for the transport mechanism. In that case the ratio \( T_M/K_T \) probably would be so small that it may be omitted for all practical purposes. An alternative explanation might be the effective inhibition of the secretion by plasma protein binding. Although we cannot rule out this possibility, the fact that the protein binding of alclofenac is extensive but labile seems to give evidence against it. So, it is quite unlikely that the situation as defined by equation 8.2 prevails. There is, however, another way in which a tubular secretion process might be concealed in the overall pictures as given in figure 8.5. At this stage of the discussion reference to our ibuprofen study (chapter 5) is pertinent. In case of ibuprofen we found clear indications for tubular secretion. The transport maximum was very low, but also the linear part of the renal clearance was quantitatively very small. Alclofenac has a carboxylic acid group in common with ibuprofen. This is one of the prerequisites for tubular secretion, but when other factors (like an amino function) are lacking, the secretion mechanism appears to have only limited capacity. In this respect there seems no need for assuming differences between alclofenac and ibuprofen, but there is another very important difference: the linear part of the renal clearance of alclofenac is much higher that that of ibuprofen. This may cause that a possible secretion mechanism vanishes into a quantitatively much larger filtration. As a consequence the actual situation would best be described by the other extreme of equation 8.1, prevailing when \( C \gg K_T \):

\[
\frac{dQ_r}{dt} = k_{Cr} C_p + T_M
\]  

(8.3)

Also this equation predicts a linear relationship between renal excretion rate and plasma concentration, but the straight line fitting this relationship will not pass through the origin. The fact that the regression lines as depicted in figure 8.5 often cross the \( dQ_r/dt \)-axis above the origin may be meaningful, but the difference between the actual intercepts and the origin

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cannot be made statistically significant on basis of our data alone. Never­
theless we may conclude that for alclofenac tubular secretion, if present, 
has only a very limited capacity (less than 5 μg/min).

**Chronic administration**
The short halflife of alclofenac excludes the possibility of accumulation in 
a dosage regimen of one dose three times daily. An advantage of this is 
that dangerously high levels will not easily be reached, but a disadvantage 
may be that it will be impossible to obtain a steady level of the drug in 
plasma. For the moment it is not known whether fluctuations in the 
plasma level affect the therapeutic effectiveness of antiinflammatory drugs 
adversely or not, but it is hardly conceivable that it would not be of 
advantage to have a real steady state as far as drug concentration in plasma 
is concerned. One of the volunteers participating in this study took one 
Mirvan® tablet thrice daily for two weeks. The result of the measurements 
of the plasma concentration during this period is shown in fig. 8.6.

![Figure 8.6](image)

*Figure 8.6*  
**Plasma concentration during repeated administration (500 mg, 3 dd) of alclofenac.**  
The curve is simulated on basis of the parameters obtained after a single dose. The 
plasma levels are very fluctuating and no accumulation occurs.
Obviously very large fluctuations and practically no accumulation occur. The rate of elimination at the end of the two weeks period is exactly the same as at the start of the experiment. No variations in renal excretion, other than caused by the factors discussed above, were seen. The curves drawn in the figure are simulated by computer on basis of constant pharmacokinetic behaviour and constant biological availability. The theoretical curve matches the experimental data reasonably well, but the picture is somewhat flattering since the time axis is relatively short, so that deviations are not clearly visible. Closer examination suggests that appreciable variations in bioavailability and absorption rate occur. In view of the reasonably fitting overall picture, however, we tend to assume that it will be possible to design directives for optimal individual dosage regimens. A first approach in this direction can be found in the paper by Strolin-Benedetti et al. (1973).

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Fellman, N., Praxis 60, 882 (1971).
CHAPTER 9 PHARMACOKINETICS OF FLUFENAMIC AND MEFENAMIC ACID IN MAN

INTRODUCTION

Flufenamic acid*, N-(3-trifluoromethylphenyl)anthranilic acid, and mefenamic acid**, N-(2,3-dimethylphenyl)anthranilic acid, are representatives of a large group of N-phenylanthranilic acid derivatives (fenamates) that have been synthesized and tested for antiinflammatory activity. These two fenamates appear to have some analgesic and antipyretic effect, but especially their antiinflammatory effectiveness has drawn the attention. Numerous references concerning this matter may be found in a monograph edited by Hume Kendall (1967), in which results of experimental laboratory studies as well as clinical trials are reported. The antiinflammatory effect of mefenamic acid is claimed to be equivalent or slightly superior to acetylsalicylic acid, whereas flufenamic acid seems to be 2-3 times as potent. Several types of side-effects may occur during therapy with fenamates. As for all antiinflammatory drugs gastro-intestinal disturbances occur. Headache and skin rashes are occasionally met. Also some blood disorders and liver function disturbances have been reported in association with the use of fenamates (for a review of the side-effects, see Prescott, 1972). In the Netherlands mefenamic acid is marketed, flufenamic acid is not.

The present study was undertaken in order to obtain more insight into the pharmacokinetic behaviour of the two fenamates in man. From a theoretical point of view the drugs were especially interesting because of their extremely low water solubility, which should be expected to cause difficulties in the absorption process. From a more practical point of view possible implications of a suspected enterohepatic circulation on the profile of the plasma curve were interesting.

PHARMACOKINETICS

Little information on the pharmacokinetics of flufenamic and mefenamic acid in man can be found in literature. Only in a paper by Glazko (1967) some plasma levels in man after oral intake of these fenamates are given. Although the data clearly deviate from one-compartment kinetics, it seems that the major part of the plasma decay curve is monophasic. Only when

* Arlef®, reg. trademark Parke-Davis
** Ponstan®, reg. trademark Parke-Davis
very low concentrations are reached the elimination rate appears to slow
down, suggesting that at least two compartments are involved. From the
data of Glazko we estimate the relevant halflives for elimination roughly at
2–3 hrs for flufenamic acid and somewhat lower for mefenamic acid. The
most predominant features of the kinetics of the fenamates seem to be a
strong protein binding and an extensive biliary excretion and reabsorption
(enterohepatic circulation) as evidenced by animal experiments (Glazko,
1967).

MATERIALS AND METHODS

Drugs
Flufenamic acid and mefenamic acid in a pure form were obtained from
Parke-Davis Bornem, Belgium, which is gratefully acknowledged. Mefenamic
acid tablets (Ponstan®) were commercially obtained as they are marketed
in the Netherlands.

Drug administration
Flufenamic acid was administered to 8 young, healthy, male volunteers in
the form of soft gelatin capsules containing 200 mg of pure substance. The
subjects were fasting overnight prior to the drug ingestion and for an
additional 3 hrs thereafter and took the capsule with 100 ml of water.
Mefenamic acid was first administered to 8 young, healthy, male volun­
teeers who had fasted overnight and remained without food for an addi­
tional 3 hrs after drug intake. In view of the low plasma levels obtained we
judged it worthwhile to repeat the experiment with the same volunteers
after they had taken breakfast. Six of the eight volunteers from the first
part of the study were able to participate in this experiment. The subjects
were instructed to have breakfast (in the English way) 0.5 to 1 hr before
drug administration, but the breakfast was not standardized. The drug was
administered in a 1 g dose (4 tablets Ponstan®) together with about
100 ml of water.
Preparation of samples, fluorometric analysis of flufenamic acid and gas
chromatographic analysis of mefenamic acid were as described in
chapter 2.

RESULTS

Flufenamic acid
Figure 9.1 shows the individual plasma curves obtained. The data are
plotted on a linear scale, since apparently they do not obey simple pharmacokinetics. No acceptable computer fit could be obtained, neither on basis of a one-compartment model nor on basis of a two-compartment model. Maximal plasma levels are obtained usually in about 1.5 hrs, but in two subjects (PK and MB) this took much longer times. The peak heights show a large interindividual variation. This might be the result of differences in biological availability. In table 9.1 some indicative parameters are
TABLE 9.1

Some parameters for flufenamic acid plasma curves (D=200 mg, capsule)

<table>
<thead>
<tr>
<th>Subject</th>
<th>$t_{\text{max}}$ (min)</th>
<th>$C_{\text{max}}$ (mg/1)</th>
<th>AUC (mg. hr/1)</th>
<th>$k_{C\text{el}}/F$ (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB</td>
<td>90</td>
<td>5.9</td>
<td>25.1</td>
<td>133</td>
</tr>
<tr>
<td>JBr</td>
<td>90</td>
<td>20.2</td>
<td>43.8</td>
<td>76</td>
</tr>
<tr>
<td>MBr</td>
<td>290</td>
<td>10.5</td>
<td>54.4</td>
<td>61</td>
</tr>
<tr>
<td>HH</td>
<td>80</td>
<td>8.5</td>
<td>39.7</td>
<td>84</td>
</tr>
<tr>
<td>BK</td>
<td>50</td>
<td>10.6</td>
<td>37.9</td>
<td>88</td>
</tr>
<tr>
<td>PK</td>
<td>170</td>
<td>5.4</td>
<td>35.1</td>
<td>95</td>
</tr>
<tr>
<td>FR</td>
<td>90</td>
<td>15.4</td>
<td>44.3</td>
<td>75</td>
</tr>
<tr>
<td>DZi</td>
<td>90</td>
<td>13.0</td>
<td>38.6</td>
<td>86</td>
</tr>
</tbody>
</table>

1. All volunteers are male, age 20-26 yr, body weight 60-80 kg.

2. $t_{\text{max}}$ and $C_{\text{max}}$ refer to the time and the concentration of the measured plasma peak.

listed and also the area under the curve is given. This area has graphically been determined from the linear plot of the plasma concentration versus time. From the last measured concentration point the residual area (to infinity) has been estimated according to the following obvious relationship.

$$AUC_{0-\infty} = AUC_{0-t'} + \int_{t'}^{\infty} C \, dt$$

(9.1)

where

- $AUC$ = area under the linear plasma curve = $\int C \, dt$
- $C$ = plasma concentration
- $t$ = time

and the suffixes denote the time range over which the area is estimated.

Under the assumption that from $t'$ the decay of the plasma concentration is governed by a single time constant $\tau_{\text{el}}$, equation 9.1 simplifies to:
\[
AUC_{0-\infty} = AUC_{0-t'} + C_{t'} \tau_{el}
\]

(9.2)

where \( C_{t'} \) = plasma concentration at time \( t' \)

This equation, known as Dost's law of corresponding areas (Dost, 1968), allows (under the condition mentioned) estimation of the AUC, which has a clear pharmacokinetic meaning as long as linear kinetics prevail, viz.

\[
AUC = \frac{FD}{k_{Cel}}
\]

(9.3)

(cf. chapter 4)

For our analysis we assumed that the ultimate slope of the semilogarithmic plasma curve corresponded to \( \tau_{el} \). Of course large mistakes can be made in this estimation, but since the major part of the plasma curve was graphically determined, we may expect the relative error introduced in the total area by possibly wrong estimates of \( \tau_{el} \) not to exceed some 10% (even under the most unfavourable circumstances). From the AUC we calculated the values of \( k_{Cel}/F \), which turn out to be in the range of 60–130 ml/min. Although these are only rough estimates and although the biological availability \( F \) is unknown, it appears that the total body clearance of flufenamic acid is low. Especially in view of the fact that the estimated values may be still too high when absorption is incomplete, we may assume the clearance constant for flufenamic acid to be in the same order of magnitude as found for ibuprofen and alclofenac. Presumably also the volume of distribution is low. It is impossible to calculate a steady state volume on basis of our data, but the relatively high plasma levels found in several cases, suggest that at least the volume of the central compartment will not exceed some 15 l and may be much smaller. In view of the strong protein binding reported by Glazko (1967) this is not surprising. The small distribution volume also explains the initially rapid decrease of the plasma level, despite the low total body clearance. As far as renal excretion of unchanged drug is concerned, we were unable to find more than traces of flufenamic acid in urine.

**Mefenamic acid**

The pharmacokinetic pattern of mefenamic acid is similar to that of flufenamic acid in that it is irregular and cannot be analysed according to simple compartment models. Also its renal excretion is negligible. When
taken after an overnight fast the drug gives remarkably low plasma levels. Since we considered it possible that the absorption would improve when the gastro-intestinal motility was enhanced, we administered the drug to the same volunteers 0.5—1 hr after they had taken breakfast. The results are exemplified in figure 9.2 and some parameters for each individual are listed in table 9.2. The area under the curve again was calculated as outlined in the foregoing paragraph. It may be noted that the AUC is much smaller for mefenamic acid than for flufenamic acid when the dose ratio (1000 : 200) is taken into account. From equation 9.3 it can be seen that two factors may cause such a difference: a larger $k_{Ce}$ and/or a smaller $F$.  

![Figure 9.2](image)

**Figure 9.2**

*Some typical examples of plasma curves after oral administration of mefenamic acid (1000 mg, 4 tablets Ponstan®). The same remarks as for figure 9.1 can be made. Note that much higher levels were obtained when the subjects had breakfasted than when they were fasting. See text for discussion of this phenomenon.*
TABLE 9.2

Indicative parameters for mefenamic acid (1 g) administered to fasting (f) or not fasting (n) subjects.

<table>
<thead>
<tr>
<th>Subject</th>
<th>t_{max} (mg/1)</th>
<th>C_{max} (mg/1)</th>
<th>AUC (mg.hr/1)</th>
<th>k_{Cel}/F (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB</td>
<td>270</td>
<td>3.0</td>
<td>18.2</td>
<td>920</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>5.9</td>
<td>46.7</td>
<td>360</td>
</tr>
<tr>
<td>JBo</td>
<td>270</td>
<td>4.2</td>
<td>28.7</td>
<td>580</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>12.8</td>
<td>56.5</td>
<td>295</td>
</tr>
<tr>
<td>BK</td>
<td>260</td>
<td>4.8</td>
<td>24.1</td>
<td>690</td>
</tr>
<tr>
<td></td>
<td>265</td>
<td>10.3</td>
<td>34.6</td>
<td>480</td>
</tr>
<tr>
<td>PK</td>
<td>110</td>
<td>3.7</td>
<td>23.1</td>
<td>720</td>
</tr>
<tr>
<td></td>
<td>115; 265</td>
<td>9.2; 10.3</td>
<td>49.4</td>
<td>340</td>
</tr>
<tr>
<td>TS</td>
<td>260</td>
<td>14.1</td>
<td>48.6</td>
<td>345</td>
</tr>
<tr>
<td></td>
<td>170</td>
<td>17.3</td>
<td>62.7</td>
<td>265</td>
</tr>
<tr>
<td>RSo</td>
<td>60</td>
<td>8.3</td>
<td>29.1</td>
<td>575</td>
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<tr>
<td></td>
<td>180</td>
<td>25.1</td>
<td>75.4</td>
<td>220</td>
</tr>
<tr>
<td>HH</td>
<td>195</td>
<td>14.0</td>
<td>38.6</td>
<td>430</td>
</tr>
<tr>
<td>FR</td>
<td>195</td>
<td>5.3</td>
<td>26.9</td>
<td>620</td>
</tr>
</tbody>
</table>

1. All volunteers are male, age 20-26 yr, bodyweight 60-80 kg.
2. t_{max} and C_{max} refer to the time and the concentration of the measured plasma peak.
* plasma curve with two peaks.

On basis of our data it is impossible to discriminate between these two possibilities. Nevertheless, in view of the variability of the area under the mefenamic acid curve we assume that the biological availability of this drug from the tablets usually is low. As far as the clearance constant is concerned we would expect values in the same order of magnitude as for flufenamic acid, since also mefenamic acid is strongly protein bound (Glazko, 1967; Terada et al., 1974) and since also its initial rate of elimination is comparable with that of flufenamic acid. Therefore, it seems that
differences in the clearance constant can hardly be large enough to explain
the striking differences between the AUC's of the two fenamates. Most
likely, the difference is caused primarily by differences in F.

DISCUSSION

The pharmacokinetic pattern of the fenamates is very irregular as com­
pared to the other arylacetic acid derivatives studied. We assume that this
is related to the rather extreme physicochemical properties of these drugs.
Both drugs are only very slightly water soluble, but there is quite a
difference between the two. Whereas the solubility of flufenamic acid in an
aqueous buffer of pH 6 is about 0.1 mg/ml, the solubility of mefenamic
acid in the same solvent is not more than 0.004 mg/ml (Winder, 1967). It
is very well conceivable that such a low water solubility is a limiting factor
in the process of absorption from the gastro-intestinal tract. In this respect
flufenamic acid has somewhat more favourable properties. The irregular
absorption of the fenamates probably is directly related to the low solubil­
ity. Also the fact that our data suggest that flufenamic is much better
absorbed than mefenamic acid, is compatible with these physicochemical
characteristics. One might argue that this comparison is not fair since we
administered flufenamic acid in capsules and mefenamic acid in the form
of commercial tablets. In preliminary trials, however, we used a suspension
of crushed tablet material. In these trials the absorption appeared to be
even worse than in case of intact tablets. So it seems that the tablet
formulation is not a deteriorating factor in this respect. Obviously also
the elimination behaviour of the two fenamates is very irregular. Most prob­
ably this is caused by an enterohepatic circulation. In animal experiments
Glazko (1967) found strong indications that the fenamates are excreted
into bile and then partly reabsorbed from the intestine. In our investiga­
tions we observed in several cases that the plasma level of mefenamic acid
24 hrs after administration was higher than the level at 10 hrs after intake.
This phenomenon, which was never seen for flufenamic acid, suggests a
enterohepatic circulation in man indeed. Another explanation seems
hardly possible. It should be noticed that this implies that the estimated
area under the curve may be flattering in the sense that part of the drug in
the body contributes twice to the total area: first directly after absorption
and then again after biliary excretion and reabsorption. To avoid over­
estimation of the AUC as far as possible we extrapolated the area from
t = 10 hrs, without taking into account the higher plasma levels after
24 hrs. Nevertheless, it cannot be excluded that already during the first 10
hrs enterohepatic circulation takes place to such a degree that it increases the AUC substantially. The improvement of mefenamic acid absorption by the presence of food in the gastro-intestinal tract may be related to an increase of the bile flow. It is very well conceivable that the bile can emulsify the drug, thereby making it more suitable for absorption. A similar phenomenon has been reported for dipyridamole (Persantin®) by Mellinger and Bohorofoush (1966). These authors concluded that the plasma level of dipyridamole during chronic administration exhibits more fluctuations by the intake of food than by the intake of a new dose. We suppose that in case of chronic administration of mefenamic acid the same effects will be observed. There is another possibility that should be considered in case of drugs with very low water solubility and high lipophilicity, viz. absorption via the lymphatic system. In order to investigate this possibility, more specific (animal) experiments such as described by Kilian (1973) are needed.

Of the drugs discussed in this chapter only one (mefenamic acid) is marketed in the Netherlands, and it is that very drug that exhibits very pronounced absorption difficulties. Flufenamic acid appears to yield usually rather high plasma levels although the variability of these suggests large variations in biological availability. Mefenamic acid, however, gives lower plasma levels (although a higher dose is administered), which seems to be caused primarily by a low bioavailability. Taking into account that in several systems that are regarded as indicative for antinflammatory potency mefenamic acid is less active than flufenamic acid (see e.g. Winder, 1967, Terada et al., 1972), we tend to conclude that for antinflammatory therapy mefenamic acid must be clearly inferior to flufenamic acid. We do not know of course what the concentrations of the drugs at their site of action is, but the superiority of flufenamic acid over mefenamic acid seems to be confirmed by clinical experience (see introduction). On the other hand, as indicated already, both drugs suffer from a variety of side-effects and for the moment it is questionable if either of the two is a useful addition to the therapeutic possibilities.

Finally, it seems worthwhile to study the pharmacokinetic profile of mefenamic acid after administration of the drug in the form of a salt, for instance the sodium salt, which is freely soluble in water (Winder, 1967). Since the absorption of mefenamic acid probably is limited by its dissolution in the gastro-intestinal fluid, the absorption might be improved by administering the salt form. Such an improvement has been described for instance by Breimer (1974) for some barbituric acid derivatives.
NOTE added in proof:

After completion of this chapter Angelucci et al. (J. Pharm. Sci. 65, 455, 1976) reported measurements of the plasma concentration-time curves of flufenamic acid in dogs and humans after administration of soft and hard gelatin capsules. Although the plasma concentration is measured at only 3 different times after administration their data suggest a higher biological availability of the drug from the soft gelatin capsules.

REFERENCES

CHAPTER 10 PHARMACOKINETICS OF PYRAZOLONE DERIVATIVES IN MAN

A. GENERAL INTRODUCTION*

In search of effective synthetic substitutes for quinine Knorr in 1883 obtained more or less by accident a compound for which later the generic name phenazone was proposed. Soon this compound appeared to exhibit interesting antipyretic properties. Since this was the primary effect aimed at, phenazone was put on the market as soon as it was established that the compound had a reasonably low toxicity. Under the suggestive name Antipyrine (at that time a trade name) it became available on the German pharmaceutical market in 1888. In later years it became clear that phenazone also had useful analgesic and antiphlogistic activities. The drug is used up to now, but almost exclusively in combination preparations. More important, however, is the fact that in the hands of the early pharmaceutical chemists phenazone appeared to be an excellent starting material for the synthesis of a whole series of useful and effective antipyretic, analgesic and antiphlogistic compounds. These substances usually are designated as pyrazolone-derivatives, according to the name given by Knorr to the substance from which phenazone is derived by N-methylation ('pyrazolone' is 1-phenyl-3-methyl-2-pyrazoline-5-one). Especially the carbon atom at the 4-position in phenazone has been subject to substitution during the development of pyrazolone derivatives. Before going into this in some detail we have to define the nomenclature we use, since unfortunately some of the common, generic names are quite ambiguous and not systematically derived. In this chapter all names are derived from phenazone as the basic structure and for the substituents both position and chemical identity are indicated. Table 10.1 contains the nomenclature used in this chapter together with frequently used synonyms (including some trade names) and a more systematic chemical designation according to IUPAC rules (see also figure 10.1). The substitution reactions that were carried out at the 4-position of phenazone were quite diverse, but can be divided in two major groups. The first relatively small series is that of the 4-alkyl substituted phenazones. If we restrict this introduction to the drugs that are relevant for our study, we should mention here one deriv-

* The history of the development of pyrazolone derivatives was shortly reviewed by Issekutz (1971). A general survey of chemical, physical and pharmacological properties of all pyrazolone derivatives was given by Krohs (1965).
<table>
<thead>
<tr>
<th>Nomenclature of pyrazolone derivatives</th>
<th>Frequently used synonyms</th>
<th>IUPAC nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>This chapter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>phenazone</td>
<td>antipyrin</td>
<td>1-phenyl-2, 3-dimethyl-3-pyrazolin-5-one</td>
</tr>
<tr>
<td>4-isopropylphenazone</td>
<td>propyphenazone</td>
<td>1-phenyl-2, 3-dimethyl-4-isopropyl-3-pyrazolin-5-one</td>
</tr>
<tr>
<td>4-aminoantipyrin</td>
<td>monomethylaminophenazone</td>
<td>1-phenyl-2, 3-dimethyl-4-methylamino-3-pyrazolin-5-one</td>
</tr>
<tr>
<td>4-methylaminophenazone</td>
<td>aminophenazone</td>
<td>1-phenyl-2, 3-dimethyl-4-dimethylamino-3-pyrazolin-5-one</td>
</tr>
<tr>
<td>4-isopropylaminophenazone</td>
<td>isopropyldiaminoantipyrin</td>
<td>1-phenyl-2, 3-dimethyl-4-isopropylamino-3-pyrazolin-5-one</td>
</tr>
<tr>
<td>4-methylaminophenazone-4-methanesulfonate</td>
<td>noramidopyrinemethanesulfonate</td>
<td>1-phenyl-2, 3-dimethyl-3-pyrazolin-5-one-4-methylaminomethanesulfonate</td>
</tr>
</tbody>
</table>
ative: 4-isopropylphenazone, synthesized in 1931. The second, much more important, series of phenazone derivatives is based upon 4-aminophenazone. Soon after the discovery of phenazone it was established that the introduction of an amino-function at the 4-position led to a substantial increase in activity. As early as 1897 the drug 4-dimethylaminophenazone was placed on the market under the trade name Pyramidon®. In order to obtain a 4-dimethylaminophenazone modification that was suitable for parenteral administration, 4-methylaminophenazone-4-methanesulfonate (Novalgin®) was developed in 1922. 20 Years later 4-isopropylaminophenazone was synthesized. It should be stressed that the drugs mentioned here are only a few out of a large number of drugs that have been developed and even clinically tested and applicated (for a more complete survey see Frohs, 1965).

Numerous studies have been published concerning the pharmacology of the phenazone derivatives. All compounds have antipyretic, analgesic and antiinflammatory activities, but to a variable extent. Taking phenazone as a standard one should regard the fact that, although it exerts all three effects to such a degree that is was considered a very promising compound at the time of its development, today the standard is so high that phenazone should be classified as a drug with only very moderate activity.

Figure 10.1
Structural formulas of the phenazone derivatives discussed in this chapter.
The 4-aminophenazone series, however, is more active. Especially 4-dimethylaminophenazone (Pyramidon®) has been the drug of choice for treatment of fever and inflammatory conditions for a long time. Its antipyretic activity justifies its application even today under certain circumstances, but without careful control use should be minimized in view of the risk of serious side-effects. The same holds true for 4-methylaminophenazone-4-methanesulfonate, which has, however, only about one tenth of the activity and acute toxicity of 4-dimethylaminophenazone (in animal experiments) but which can therefore be administered in higher doses. In this case the effects will not be brought about by the parent compound, which is not detectable in plasma, but by its metabolites 4-methylaminophenazone and (to a much smaller extent) 4-aminophenazone (Weiss et al., 1974). Therefore one might conclude that also the pharmacological activity of 4-methylaminophenazone is approximately one tenth of that of 4-dimethylaminophenazone, although no direct evidence is available.

4-Aminophenazone has not come into clinical use as an analgesic; occasionally it has been used for determination of the volume of total body water (Huckabee, 1956). Its antipyretic and analgesic action seems comparable with 4-dimethylaminophenazone (personal communications cited by Brodie and Axelrod, 1950). 4-Isopropylaminophenazone is in use up to now, but only together with phenylbutazone in combination preparations (Tomanol®). According to Schoetensack et al. (1960) the drug has reasonable antipyretic, analgesic and antiphlogistic activity.

Finally, the only alkylsubstituted derivative of phenazone mentioned in this introduction, 4-isopropylphenazone, appeared to have effects comparable with 4-dimethylaminophenazone (Fromherz, 1937; Orestano, 1935; Bauer, 1959). Today it is a component of several popular combination preparations (eg. Saridon®), but it is doubtful whether the drug in the low doses employed contributes significantly to the effect of the combination.

A large number of investigations has been devoted to establishing side-effects and toxicity of the phenazone derivatives. An excellent review concerning this matter with special reference to the human situation is available in the literature (Prescott, 1972). Phenazone and 4-isopropylphenazone are relatively safe drugs, although occasionally hypersensitivity reactions, usually in the form of skin rashes, may occur. This type of toxicity has been described for the aminoderivatives as well, but much more dangerous complications are to be feared when using this class of compounds. The complications in question are the result of bone marrow depression. Especially agranulocytosis, with a high mortality, is a notorious hazard of therapy with 4-dimethylaminophenazone (Pyramidon®).
and its sulfonic acid derivative (Novalgin®) (Hartl, 1965; Huguley, 1964; Sadusk, 1965). Most probably the pathogenesis of this type of agranulocytosis depends upon immunological mechanisms and there is evidence that the phenazone nucleus is a primary determinant in this respect (Thierfelder et al., 1964; Magis et al., 1968). However, direct evidence of agranulocytosis occurring in association with the use of phenazone or 4-isopropylphenazone has never been reported. Since on the other hand the causal relationship between the use of 4-dimethylaminophenazone (and 4-methylaminophenazone-4-methanesulfonate) and agranulocytosis is a well-established fact, one tends to regard the 4-amino group as an important secondary characteristic. The incidence of agranulocytosis by these drugs is not exactly known, but estimations range from 0.007 to 1%, with a mortality rate of about 75% (cf. for instance Huguley, 1964; Hartl, 1965). The agranulocytosis may develop suddenly in patients who have taken the drug for some time without any problems and in a sensitive individual disappearance of granulocytes from the blood may be complete within 6 to 24 hours after drug intake. The risk of agranulocytosis makes 4-dimethylaminophenazone and 4-methylaminophenazone-4-methanesulfonate hazardous drugs that should be available only on prescription and only under strict medical control. This applies also to their use in combination preparations. For instance the presence of 4-methylaminophenazone-4-methanesulfonate in combination with a spasmolytic in popular preparations like Buscopan® compositum and Baralgin® may carry the risk that this potentially dangerous drug is prescribed by physicians who are unaware of the exact composition of the preparations. In general one should prefer the administration of the spasmolytic alone, or together with a safe analgesic in a separate form.

Although our study is confined to the phenazone derivatives in a strict sense, we mention here two other drugs that can be regarded as pyrazolone derivatives as well, viz. phenylbutazone and oxyphenbutazone. In these compounds a second carbonyl group is introduced in the pyrazole nucleus (see figure 10.1), which gives the drugs a clear acidic character, contrary to the phenazone derivatives that are all more or less basic. It is interesting to note here the analogy with the newer non-steroidal antiinflammatories, which as a rule have a free carboxylic acid group. Since the introduction of phenylbutazone (Butazolidin®) towards the end of the forties its excellent antiinflammatory (and antipyretic) properties have been established in many studies (Von Rechenberg, 1961). The same holds true for oxyphenbutazone (Tanderil®) which is a metabolite of phenylbutazone. Unfortunately these drugs show quite a large incidence of serious side-effects and
the same type of blood disorders as with 4-aminophenazone derivatives can be encountered, but several other dangerous complications as well (Prescott, 1972).

The present pharmacokinetic study in man was undertaken in order to obtain insight in the influence of substitution in the 4-position of the phenazone nucleus on the nature and the rate of the elimination of the phenazone derivatives in man and on the other kinetic parameters. The results of these investigations are meaningful from two different points of view. Firstly, there is a good chance of getting some general ideas on structure-pharmacokinetics relationships and secondly, the pharmacokinetic data will be helpful for interpreting the time course of the effects and for establishing optimal dosage schedules especially for those compounds that are therapeutically employed. This may be the more important as the disposition of several of the commonly used drugs mentioned in this introduction was practically totally unknown (e.g. 4-isopropylphenazone and 4-isopropylaminophenazone). This chapter is divided into two parts: the first deals with the comparative pharmacokinetics of phenazone and 4-isopropylphenazone, the second with a comparison of 4-dimethylaminophenazone, 4-methylaminophenazone, 4-aminophenazone and 4-isopropylaminophenazone. Concerning phenazone and 4-dimethylaminophenazone a lot of kinetic studies have appeared in literature already, some of which will be referred to in the following parts of this chapter, and in our investigations these two drugs were included mainly for reference purposes.

B. PHENAZONE AND 4-ISOPROPYLPHENAZONE

INTRODUCTION

The fate of phenazone in the human organism has been extensively studied. Already in 1950 Brodie and Axelrod reported that phenazone after oral administration is completely absorbed and then evenly distributed throughout the body water. Therefore phenazone might be used for determining the volume of total body water (Soberman et al., 1949). They also noted a remarkably slow elimination of the drug in man (half-life in the order of 15 hrs). The fact that only about 5% of the dose was excreted unchanged in urine indicated that most of it is eliminated by metabolic transformation. Already in this first paper on human kinetics of phenazone Brodie and Axelrod identified the major metabolite: 30 to 40% of the dose is oxidized to 4-hydroxyphenazone, which is quickly and com-
pletely conjugated with glucuronic acid and possibly with sulfuric acid. Three other metabolites have been found in urine, largely in a conjugated form: N-demethylphenazone (Schüppel, 1966) and further a hydroxymethylphenazone and a dihydrodiol of phenazone (Stafford et al., 1974). These last two metabolites suggest that the biotransformation of phenazone proceeds via the epoxide-dihydrodiol pathway. Phenazone is only slightly bound to plasma proteins (about 5%) and together with the fact that it is almost completely metabolized, this has led to speculations on the utility of the drug's elimination as a measure of drug metabolism by oxidative pathways in man. Vesell and Page (1968) indicated that the halflife of phenazone (5.1 to 16.7 hrs with an average value of 10.9 hrs in their study) was genetically controlled. Another interesting argument for the suitability of phenazone for evaluation of metabolic capacity lies in the clear correlation that is found between the rate of plasma elimination of phenazone and the rate of appearance in urine of the metabolite 4-hydroxyphenazone (Huffman et al., 1974; Vesell et al., 1975). What is aimed at, however, is the possibility to predict the rate of elimination of other drugs on basis of the measured elimination of a standard compound. Now it is clear that phenazone is eliminated by oxidative processes and its predictive value should be restricted to other drugs that also are largely oxidated. The fact that the elimination rate of phenazone shows such a tremendous interindividual variation, but remains remarkably constant in the same individual examined over a period of more than 1 year, seems to suggest that it provides a reasonable reflection of the oxidative metabolic capacity of each individual (Davies et al., 1973; Davies and Thorgerisson, 1971a). Studies to confirm this, however, have seldom been unambiguous. Whereas Vesell and Page (1968) were unable to find any correlation between the halflives of phenazone and phenylbutazone in 28 twins, Davies et al. (1973) found a significant correlation between these halflives in 8 subjects. An equally significant correlation was found with the halflife of oxyphenbutazone (Davies and Thorgerisson, 1971b). Smith and Rawlins (1974) reached again the conclusion that the halflife of phenazone could not be used to predict the elimination rate of phenylbutazone. In a comparative study on the elimination of phenazone, glutethimide, amobarbital and sulfinpyrazone, the halflife of phenazone appeared to be not correlated with that of any of the other 3 drugs (Kadar et al., 1973). The same negative result was found by Vesell et al. (1975) in a comparison of phenazone, 4-dimethylaminophenazone and phenacetin. In view of these results the predictive value of the halflife of phenazone seems to be very limited and not practically useful. Nevertheless one must
assume that it gives some indication of the metabolic status, especially since phenazone clearance, as expected, is not influenced by renal failure (Lichter et al., 1973). Evidence in favour of this assumption can be found indeed. Welch et al. (1975) showed a largely decreased half-life of phenazone in patients treated with anticonvulsants for more than 2 months. The same authors demonstrated that the elimination of phenazone can very well be evaluated from measurement of the decay of the concentration of phenazone in saliva (see also Vesell et al., 1975). There are several factors, known to alter hepatic function, that influence phenazone clearance in man. For instance phenobarbital treatment increases the rate of elimination of phenazone significantly (Vesell and Page, 1969; Kampffmeyer, 1971). The degree of this increase seems to be under genetic control (Vesell and Page, 1969). Thyroid status is another factor: the half-life of phenazone is much shorter in hyperthyroid than in hypothyroid patients (Eichelbaum et al., 1974). Phenazone metabolism is also affected by age, alcohol consumption, caffeine and smoking (Vestal et al., 1975; Vesell and Passananti, 1973), by physical stress (Swartz et al., 1974) and by fever (Elin et al., 1975). The half-life of phenazone is decreased immediately by administration of hydrocortisone (Breckenridge et al., 1972) but prolonged under the influence of several other drugs (disulfiram, prazepam, allopurinol, nortriptyline, tetrahydrocannabinol and levodopa; Vesell and Passananti, 1973). On the other hand phenazone itself has enzyme-inducing properties (Breckenridge et al., 1970), leading to increased glucuronidation of bilirubin (Orme et al., 1974) and to shortening of its own half-life and fall in its own steady state levels (Davies et al., 1974).

This short and incomplete anthology gives an indication of how much activity has been devoted to describing and interpreting the pharmacokinetics of phenazone. Curiously enough no pharmacokinetic data at all are available in literature up to now with respect to 4-isopropylphenazone. The only more or less 'kinetic' observation was reported by Bauer (1959) who found the analgesic effect of this drug in mice to be of shorter duration than that of 4-dimethylaminophenazone.

Since 4-isopropylphenazone is an extensively used drug in combination preparations we judged it worthwhile to study its pharmacokinetic behaviour in man, for instance to see if this drug might be expected to accumulate when the combinations are used. Apart from this practical interest we wondered what would be the influence of isopropyl-substitution in phenazone and what (if any) would be the correlation between the pharmacokinetic properties of phenazone and 4-isopropylphenazone.
MATERIALS AND METHODS

Phenazone and 4-isopropylphenazone were obtained from OPG, Utrecht, The Netherlands.

Preparation of samples and analytical procedure were as described in chapter 2.

Drug administration
Eleven young, healthy volunteers participated in this study. Seven of them took both phenazone and 4-isopropylphenazone. The other 4 volunteers were only disposable for one of the experiments. Prior to ingestion of the drug at nine o’clock at the day of experimentation, they were fasting overnight. After drug intake they remained without food for an additional 3 hrs. No other drugs were taken during and for a period of at least two weeks before the experiments. The period between the two experiments was at least two weeks, but in some cases much longer. Longer intervals, however, did not influence the validity of the comparison unfavourably, since the pharmacokinetic behaviour of the two drugs under study was checked to be remarkably constant within the same individual over long periods of time (about 1.5 year). Blood samples were taken from the fore arm vein at regular intervals after drug administration. Samples of every aliquot of urine voided were collected after registering time and total volume of the urine production, for a period of at least 30 hrs (in case of 4-isopropylphenazone) or 60 hrs (in case of phenazone). Phenazone was administered in a dose of 1000 or 500 mg in the form of a solution in about 100 ml of water. 4-isopropylphenazone was administered in the form of gelatin capsules containing 500 mg of the pure drug, together with about 150 ml water. Preliminary trials in which we administered 4-isopropylphenazone as powder with a glass of water were unsatisfactory since the powder was irritating to pharynx and oral cavity. The drug’s watersolubility is far too low to obtain solutions suitable for administration.

RESULTS AND DISCUSSION

As exemplified in figures 10.2 and 10.3 the pharmacokinetic behaviour of both phenazone and isopropylphenazone after oral administration can adequately be described on basis of the open one-compartment model. Looking at the curves (which are based on computer fit), especially the large difference in the rate of elimination is striking. This difference
Figure 10.2
Comparison of the plasma curves obtained after oral administration of phenazone (1000 mg) and 4-isopropylphenazone (500 mg) to the same volunteer. Note the large difference between the half-lives.

Figure 10.3
Comparison of the plasma curves after oral administration of phenazone (500 mg) and 4-isopropylphenazone (500 mg). Note the large differences in half-life and apparent volume of distribution.
### TABLE 10.2

Pharmacokinetic parameters for Phenazone and 4-isopropylphenazone.

<table>
<thead>
<tr>
<th>Subject⁴</th>
<th>V/F</th>
<th>k&lt;sub&gt;Cel/F&lt;/sub&gt; (1)</th>
<th>τ&lt;sub&gt;el&lt;/sub&gt; (hr)</th>
<th>t&lt;sub&gt;½&lt;/sub&gt; (hr)</th>
<th>τ&lt;sub&gt;a&lt;/sub&gt; (min)</th>
<th>V/F (1)</th>
<th>k&lt;sub&gt;Cel/F&lt;/sub&gt; (ml/min)</th>
<th>τ&lt;sub&gt;el&lt;/sub&gt; (min)</th>
<th>t&lt;sub&gt;½&lt;/sub&gt; (min)</th>
<th>τ&lt;sub&gt;a&lt;/sub&gt; (min)</th>
<th>lag (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA (23,60,172)</td>
<td>34</td>
<td>32</td>
<td>18.1</td>
<td>12.5</td>
<td>12</td>
<td>84</td>
<td>370</td>
<td>224</td>
<td>155</td>
<td>105</td>
<td>16</td>
</tr>
<tr>
<td>HB (22,68,175)</td>
<td>49</td>
<td>52</td>
<td>15.7</td>
<td>10.9</td>
<td>9</td>
<td>73</td>
<td>235</td>
<td>310</td>
<td>215</td>
<td>141</td>
<td>35</td>
</tr>
<tr>
<td>MB (24,80,186)</td>
<td>45</td>
<td>43</td>
<td>17.2</td>
<td>11.9</td>
<td>6</td>
<td>75</td>
<td>780</td>
<td>95</td>
<td>66</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>WB (22,81,188)</td>
<td>41</td>
<td>49</td>
<td>14.1</td>
<td>9.8</td>
<td>11</td>
<td>122</td>
<td>850</td>
<td>144</td>
<td>100</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>JE (24,90,186)</td>
<td>46</td>
<td>45</td>
<td>17.0</td>
<td>11.8</td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>KF (25,70,178)</td>
<td>31</td>
<td>28</td>
<td>18.8</td>
<td>13.0</td>
<td>26</td>
<td>85</td>
<td>750</td>
<td>113</td>
<td>78</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>LH (24,66,176)</td>
<td>31</td>
<td>30</td>
<td>17.0</td>
<td>11.8</td>
<td>7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>ML (22,51,165)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>76</td>
<td>700</td>
<td>109</td>
<td>75</td>
<td>92</td>
<td>9</td>
</tr>
<tr>
<td>MR (22,54,167)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>72</td>
<td>350</td>
<td>207</td>
<td>143</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>JV (26,75,180)</td>
<td>32</td>
<td>36</td>
<td>14.5</td>
<td>10.1</td>
<td>15</td>
<td>89</td>
<td>670</td>
<td>133</td>
<td>92</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>DZ (23,60,175)</td>
<td>39</td>
<td>50</td>
<td>13.0</td>
<td>9.0</td>
<td>42</td>
<td>42</td>
<td>370</td>
<td>114</td>
<td>79</td>
<td>29</td>
<td>10</td>
</tr>
</tbody>
</table>

1. Numbers in parentheses represent age (yr), bodyweight (kg) and height (cm); all subjects are male, except ML and MR.
2. Oral dose 1000 mg, except MB and HB (500 mg).
3. Oral dose 500 mg.
indicates that the introduction of the isopropyl group at the 4-position of phenazone influences the pharmacokinetic behaviour to a large extent. Table 10.2 shows a compilation of all relevant pharmacokinetic parameters, and it is obvious at first sight that the impression the figures give is substantiated. We will now discuss the data in terms of the single processes and at any stage compare the results for phenazone with those for 4-isopropylphenazone.

Absorption
As a rule in our study the absorption of phenazone was more rapid than that of 4-isopropylphenazone. However, it is unallowed to draw far reaching conclusions from this observation, since the drugs were not administered in the same formulation. The fact that phenazone is rapidly absorbed from a watery solution is just a confirmation of what should be expected for a drug with good water- and lipid-solubility. In case of 4-isopropylphenazone the dissolution of the drug in the gastrointestinal juice might be a rate limiting step, since it has only a very low watersolubility. Disintegration of the capsules cannot be expected to take much time, since for other drugs we found extremely high absorption rates when administered in the same capsule. Optimal fit of the experimental plasma concentration data to the one compartment model required a certain lag period in case of 4-isopropylphenazone. This also is a strong indication that before the actual absorption process becomes first order, some other step is rate limiting. The fact that by introducing this lag-time, a very good fit is obtained indeed, guarantees the validity of the estimated parameters, since obviously the lag-time is only a short induction period during which a steady concentration-gradient is established, whereafter the absorption process is merely first order. For phenazone it was unnecessary to consider the possibility of a lag-time, since this never led to an improvement of the fit. The analysis of an absorption process in terms of two parameters, the time constant for absorption and the lag-time, usually offers special difficulties with regard to the certainty of the estimated parameters. Unless many data points are available in the absorption phase one can easily imagine that a strong negative correlation exists between the lag-time and the time constant for absorption. In other words: prolonging of the lag-time can adequately be compensated by shortening of the time constant for absorption and vice versa. This applies to some extent to the estimates presented in table 10.2 for 4-isopropylphenazone. Therefore, when judging the rapidity of the absorption process one should combine the influence of both lag-time and time constant. In case of 4-isopropylphenazone our data
suggest that the actual absorption can be very fast (see for instance subject KF) but that the overall absorption process takes much more time since a preceding step (probably dissolution of the drug) is rate limiting.

**Volume of distribution**

As stated in the introduction phenazone is known to be distributed quite homogeneously over the total body water, although some binding to plasma proteins cannot be excluded. The volume of distribution for phenazine therefore can be expected to equal approximately the volume of body fluid, which is about 0.5 l per kg body weight for persons of normal build. The values of V/F in table 10.2 are certainly in this order of magnitude, which implies that the biological availability of the phenazone solution probably is near to unity. Much larger estimates for V/F are found for 4-isopropylphenazone. Several factors may be responsible for this apparent increase in distribution volume. First of all 4-isopropylphenazone is much more lipophilic than phenazone itself. Employing the chloroform-water system we found for 4-isopropylphenazone and phenazone distribution coefficients of about 150 and 30 respectively (see chapter 3). Also since its watersolubility as such is very low, 4-isopropylphenazone might be expected to have special affinity for fatty tissue components. On the other hand this might be compensated to some extent by binding to plasma proteins, the degree of which is not known but which certainly may be expected to be higher than for phenazone. Secondly the bioavailability of 4-isopropylphenazone in the form applied may be incomplete. Obviously this would cause overestimated values for V. When for instance the bioavailability F would be about 0.5 the actual volumes would have half the values listed in table 10.2, which would bring them in the same order of magnitude as found for phenazone. In view of the regular absorption process which is seen (at least after the induction period) it seems unlikely, however, that the bioavailability would be so low, especially since the drug was administered on an empty stomach. A third phenomenon therefore seems to be more plausible, viz. a substantial first-pass effect. After absorption the drug goes via the vena porta directly to the liver where already at its first passage part of the drug may be extracted from the bloodstream. This is certainly not unlikely in view of the high clearance constants and taking into account that only negligible amounts of 4-isopropylphenazone are excreted unchanged in urine (see below). This first pass effect would diminish the amount of drug that reaches the general circulation intact, thereby decreasing the effective dose. Formally this effect can be regarded as a reduction of the biological
availability. Possible implications of the first-pass effect for 4-isopropylphenazone are discussed below under 'clearance'.

Elimination
The halflives for elimination found in this study, show a large interindividual variability for both drugs. This variability is well-known for phenazone (see e.g. introduction) but for 4-isopropylphenazone not, since for this drug no data at all were available up to now. In all of the seven subjects who received both drugs, 4-isopropylphenazone was eliminated at a much higher rate than phenazone, the ratio between the halflives varying from 3.0 to 10.9 with an average of 6.85. No correlation, however, existed between the halflives found for the two drugs in the same individuals. This suggests that different metabolic routes are primarily involved in the elimination of phenazone and its 4-isopropyl derivative. The fact that the elimination of the 4-isopropyl compound is much more rapid than that of the unsubstituted substance seems to indicate that the isopropyl group is indirectly or directly involved in the biotransformation process, since the drugs have all other molecular characteristics in common. Indirectly the isopropyl group could enhance the metabolic transformation by increasing the lipid solubility and thereby facilitating penetration into the liver cells. On the other hand it is very unlikely that the penetration of the liver cells would be rate limiting for phenazone, since its distribution over the body water is definitely much faster than its elimination. Therefore this indirect influence may safely be neglected, and only a direct involvement of the isopropyl group in the metabolic process has to be considered. It is well-known that the most important metabolite of phenazone is 4-hydroxyphenazone (cf. introduction). Obviously this metabolite cannot be formed in case of 4-isopropylphenazone. Most probably metabolic attack here is directed primarily towards the isopropyl group. So it is likely that, whereas the 4-hydroxy metabolite of phenazone may be the result of epoxidation (Stafford et al., 1976), the most important metabolites of 4-isopropylphenazone will be produced by aliphatic (side chain) oxidation. The differences in rate of elimination might be (partly) explained on this basis.

With regard to the remarkably large variability in the rate of elimination for the two drugs under study, it is important to note that this variability for phenazone most probably is under genetic control (Vessel and Page, 1968). This may also be the case for 4-isopropylphenazone, but with regard to this drug also the influence of variations in hepatic bloodflow has to be taken into account. Contrary to phenazone, 4-isopropylphenazone exhibits such a large metabolic clearance that variations in the bloodflow
through the liver undoubtedly will be reflected in the metabolic clearance constant and consequently also in the halflife for elimination. This may be an additional factor contributing to the interindividual variability of the observed halflives.

As far as practical implications of our data are concerned it seems justified at this stage of the discussion to throw doubt upon the rational basis of the addition of relatively small amounts of 4-isopropylphenazone to a lot of combination preparations. Our study reveals that small doses (for instance 150 mg as in Saridon®) cannot be expected to give rise to effective blood levels for a relatively weak compound, as 4-isopropylphenazone is. Furthermore in many persons the elimination of the drug will be so rapid, that even if the peak concentration would be above the minimally effective concentration, such an effective level would only last for a short period of time. Unless a substantial synergism between the components of these combination preparations would be proven, the disadvantages of dealing with sometimes very diverse drugs simultaneously can only seldom be counterbalanced by possible advantages of lower toxicity. This is the more so, since it is very questionable whether these combinations offer any decrease of potential toxicity at all. Furthermore it has to be noticed that most drugs will not potentiate other drugs unless they reach reasonable plasma levels themselves.

**Renal clearance**

**TABLE 10.3**

Cumulative renal excretion (% of dose) of phenazone and 4-isopropylphenazone.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Phenazone</th>
<th>4-isopropylphenazone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Q_r$% (unch.)</td>
<td>$Q_r$% unch.</td>
</tr>
<tr>
<td>HA</td>
<td>6.0</td>
<td>0.05</td>
</tr>
<tr>
<td>HB</td>
<td>2.9</td>
<td>0.11</td>
</tr>
<tr>
<td>MB</td>
<td>1.7</td>
<td>0.09</td>
</tr>
<tr>
<td>WB</td>
<td>1.9</td>
<td>0.07</td>
</tr>
<tr>
<td>JE</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>KF</td>
<td>3.2</td>
<td>0.01</td>
</tr>
<tr>
<td>LH</td>
<td>3.9</td>
<td>-</td>
</tr>
<tr>
<td>JV</td>
<td>1.7</td>
<td>0.02</td>
</tr>
<tr>
<td>DZ</td>
<td>5.1</td>
<td>0.17</td>
</tr>
</tbody>
</table>

x not detectable.

"met. 1" and "met. 2" refer to metabolites discussed in the text.
As shown in table 10.3 phenazone is excreted unchanged in urine for 1.7 to 6% of the dose administered. From the detailed renal excretion graphs, an example of which is given in figure 10.4, we got the impression that the renal excretion rate fluctuated more or less proportionally to the urineflow. This was not surprising on the basis of the physicochemical properties of phenazone. Like alcofenac (chapter 8) phenazone exhibits an intermediate lipid solubility, but further phenazone has a high watersolubility.

![Renal Excretion Rate and Cumulative Renal Excretion of Phenazone](image)

**Figure 10.4**
Renal excretion rate and cumulative renal excretion of phenazone after an oral dose of 1000 mg.

With regard to renal excretion this suggests a reabsorption which is far from complete and strongly dependent on the ultimate volume of urine produced. A positive correlation between the percentage of drug excreted unchanged and the average urineflow prevails indeed. Figure 10.5 indicates that the same type of correlation is found between the renal clearance constant and the urineflow. It should be noted that unlike the percentage excreted unchanged, the renal clearance constant determined has an

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The renal clearance constant of phenazone plotted against the average urine flow. Note the positive correlation; the line is the result of the regression analysis.

absolute meaning, since its value is independent of the biological availability (cf. the discussion of the renal excretion of alclosenac in chapter 8).

4-isopropylphenazone is practically not excreted unchanged. Table 10.3 contains the relevant data concerning the renal excretion of this drug and shows that not more than about 0.1% of the dose appears in urine unchanged. Of course this is a negligible fraction of the total dose administered. No correlation at all could be found with the rate of urine production. It is interesting to note that gaschromatograms of the urine extracts obtained after 4-isopropylphenazone administration show two peaks that seem to belong to metabolites as suggested by their course as a function of time. A representative example of the estimated renal excretion rates and cumulative renal excretion of these products together with the data for the unchanged drug is given in figure 10.6. Clearly the total amount of the 'metabolites' is rather small. Table 10.3 shows that not more than about 3 percent of the dose can be accounted for by renal excretion of the drug itself and presumable metabolites. The identity of the metabolites could not be established.
It can be concluded that detailed analysis of the renal excretion of phenazone and 4-isopropylphenazone proves that for all practical purposes the elimination of these drugs may be regarded as determined by metabolic clearance alone.

**Clearance**

Phenazone has a low overall clearance constant; the values we found for k_{Cel}/F range from 25—50 ml/min. Since presumably the biological availability is practically complete, we may assume that this range also applies to the absolute values for the clearance constant. Interestingly phenazone is cleared essentially at the same rate as ibuprofen and alclofenac (see chapters 5 and 8), but because of its much larger volume of distribution a much longer half-life results. In case of 4-isopropylphenazone matters are much more complicated. From table 10.2 it is clear that the estimates for k_{Cel}/F can amount up to 850 ml/min. This implies in fact that here a substantial first-pass effect should be expected. Ideally under such circum-
stances the total-body clearance constant should be determined in an absolute sense after intravenous administration of the drug. From the ratio between this clearance constant and the hepatic blood flow the fraction of the dose extracted at its first pass through the liver can be estimated and once this is known the fraction of the dose that was absorbed can be derived. Unfortunately no pharmacokinetic parameters for 4-isopropylphenazone after intravenous administration are available. In order to get still some insight into the possible implications of the first-pass effect we will discuss this matter under the basic assumption that the whole dose administered has come to absorption. The fact that for pyrazolone derivatives absorption as a rule seems to be practically complete may be regarded as an argument in favour of this assumption. When after oral administration a drug is absorbed, it is collected in the vena porta and goes directly to the liver. The drug can leave into two directions: one is with the bloodstream unchanged out of the liver, the other is into the liver cells to be metabolized. The relative contributions of these two ways of course depend upon the ratio of their capacities, which can best be expressed as the volume of blood that flows in either direction per unit of time. As far as the metabolic route is concerned, this flow should be interpreted of course in an abstract sense, viz. as the metabolic clearance. Now the clearance constants we use are always based on measurement of plasma concentrations and should be termed plasma-clearance constants. What we need for our comparison is of course the blood clearance, since pyrazolone derivatives can exchange rapidly between plasmawater and bloodcell-water. In vitro experiments that we performed showed that 4-isopropylphenazone rapidly penetrated erythrocytes, the concentration in the cells being equal to the surrounding plasma concentration. Thus we demonstrated that the plasma concentration is equal to the total blood concentration, which implies that in this case the blood clearance is equal to the plasma clearance $k_{Cel}$. Using the symbol $f_1$ for the bloodflow through the liver, the fraction ($E$) of the amount of drug arriving through the vena porta, that is extracted at first pass obviously can be defined as:

$$E = \frac{k_{Cel}}{f_1} \quad (10.1)$$

The rest of the total amount passes through the liver unaffected, so:

$$I = 1 - \frac{k_{Cel}}{f_1} \quad (10.2)$$
where I is the fraction of the absorbed dose of drug that comes in the general circulation intact (see also Rowland, 1972). Under the assumption of complete absorption, the clearance constants given in table 10.2 for 4-isopropylphenazone can be seen as the ratio $k_{Cel}/I$. By rearrangement of equation 10.2 we obtain:

$$I = \left[ 1 + \frac{k_{Cel}}{I_f} \right]^{-1}$$

(10.3)

When in addition the hepatic bloodflow is known this equation allows calculation of I and then $k_{Cel}$ (and V). Table 10.4 gives the corrected values obtained this way for the subjects that participated in our study. Also the estimated fractions $E$ and $I$ are summarized.

For the hepatic bloodflow we used a fixed value of 1500 ml/min, since no better individual estimates were available. Nevertheless it is obvious that the corrected distribution volumes are much closer to the corresponding volumes for phenazone although definitely larger in most cases. Incomplete absorption and individually variable hepatic bloodflow are complicating factors that cannot be excluded and that may influence the data of table 10.4 to some (unknown) extent.

### TABLE 10.4

4-isopropylphenazone: V and $k_{Cel}$ corrected for first-pass effect (see text for further explanation).

<table>
<thead>
<tr>
<th>Subject</th>
<th>E</th>
<th>I</th>
<th>$V_{corr.}$ (l)</th>
<th>$k_{Cel, corr.}$ (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>0.20</td>
<td>0.80</td>
<td>67</td>
<td>295</td>
</tr>
<tr>
<td>HB</td>
<td>0.14</td>
<td>0.86</td>
<td>63</td>
<td>200</td>
</tr>
<tr>
<td>MB</td>
<td>0.34</td>
<td>0.66</td>
<td>49</td>
<td>515</td>
</tr>
<tr>
<td>WB</td>
<td>0.36</td>
<td>0.64</td>
<td>78</td>
<td>545</td>
</tr>
<tr>
<td>KF</td>
<td>0.33</td>
<td>0.67</td>
<td>56</td>
<td>505</td>
</tr>
<tr>
<td>JV</td>
<td>0.31</td>
<td>0.69</td>
<td>62</td>
<td>460</td>
</tr>
<tr>
<td>DZ</td>
<td>0.20</td>
<td>0.80</td>
<td>34</td>
<td>295</td>
</tr>
</tbody>
</table>

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C. 4-AMINOPHENAZONE AND ITS DERIVATIVES

INTRODUCTION

The elimination of 4-aminophenazone derivatives from the body appears to proceed by a common pathway with several consecutive metabolic steps. Alkylated 4-aminophenazone derivatives are first fully dealkylated and then for a small part excreted as such and for a major part acetylated and excreted in urine as 4-acetylaminophenazone. This pathway, however, does not account for the whole dose administered and it has to be assumed that another way, perhaps involving cleavage of the pyrazolone ring, can take care of a substantial amount of drug. Oxidative dealkylation of 4-aminophenazone derivatives has been extensively studied. As a matter of fact 4-dimethylaminophenazone is used as a test substance in many investigations on drug metabolism in vitro (see e.g Mazel, 1971). In 1950 two papers appeared on the fate of 4-dimethylaminophenazone (Pyramidon®) in man (Halberkann and Fretwurst, 1950, Brodie and Axelrod, 1950b). A small amount of the drug is excreted in urine unchanged (0—4%) and about half the dose is dealkylated to 4-aminophenazone. 4-Amino­phenazone is excreted in urine as such for 10—35% and as acetylated compound 25—70%. 4-Acetylaminophenazone is almost completely excreted in urine. The excretion products of 4-methylaminophenazone—4-methanesulfonate are qualitatively and quantitatively the same as those of 4-dimethylaminophenazone. Obviously large interindividual variation in the amounts of excretion products occurs. Apart from the metabolites mentioned Pechtold (1964) found also appreciable quantities of 4-methylaminophenazone in urine of man after ingestion of the dimethyl product. He also showed the presence of traces of rubazonic acid ('Rubazonsaure') and a more substantial amount of methylrubazonic acid (see also Preuss and Voigt, 1965). These two metabolites, formed by azo coupling of two pyrazolone rings, are responsible for the red coloured urine which is observed after intake of 4-aminophenazone derivatives. Most authors mention also the occurrence of a small amount (less than 5% of the dose) of conjugated 4-hydroxyphenazone, indicating that deamination may take place. A complete survey of all these metabolites excreted in urine after oral and rectal administration is given by Gradnik and Fleischmann (1973) and by Fleischmann (1973). The fact that the excretion patterns are very similar (also quantitatively) strongly suggests that oral and rectal adminis­tration of 4-dimethylaminophenazone are practically equivalent. Two more metabolites of 4-dimethylaminophenazone have been found in rabbit
and rat, viz. the 3-hydroxymethyl derivative and the corresponding carboxylic acid (Yoshimura et al., 1970). An intermediate in the formation of these metabolites is probably the aldehyde, which may be implicated in allergic reactions (Shimeno and Yoshimura, 1972).

After this brief survey of known metabolic routes we will shortly mention some relevant pharmacokinetic data from literature for each drug separately.

4-dimethylaminophenazone

Brodie and Axelrod found an elimination rate of 10–30% an hour for this drug. This corresponds with half-lives in the range 2.3–7 hrs. They also showed absorption after oral administration to be near to complete. The drug is fairly evenly distributed throughout the body water, although some binding to plasma proteins occurs (about 15%). The concentration in the bloodcells is somewhat below the plasma concentration (see also Windorfer et al., 1973). The halflife of 4-dimethylaminophenazone can also be determined by measuring saliva concentrations as a function of time (Vesell et al., 1975). Saliva concentrations are definitely lower than plasma concentrations because of the binding of the drug to proteins in plasma. This binding, however, is rapidly reversible and relatively constant over the concentration range studied, so that the profile of the saliva concentration curve is parallel to that of the plasma curve. Vesell reported half-lives for 4-dimethylaminophenazone ranging from 1.1 to 4.5 hrs, with a mean value of 2.7 ± 0.3 hrs. The values that may be derived from data by Kaneo et al. (1973) fall within the same range. A very interesting approach to the assessment of 4-dimethylaminophenazone metabolism in man is given by Hepner and Vessel (1974). By using C14 labels in the dimethylamino-group they suggest that breath analysis to measure output of radioactive carbon dioxide provides useful information about hepatic metabolism. Phenobarbital or disulfiram pretreatment led to a significant increase and decrease respectively of the carbon dioxide output, which was also strongly diminished in case of portal cirrhosis. In this context it is interesting to note the results on demethylase activity established in human liver biopsies by Gold and Ziegler (1973). The demethylase activity appeared not to be influenced by several disease states, but decreased only upon severe tissue damage. This suggests that a prolonged half-life of 4-dimethylaminophenazone in many cases will be caused by decreased accessibility of the enzyme compartment, rather than that it would reflect alterations in 'intrinsic' hepatic clearance. Gold and Ziegler did find highest demethylase activity in patients exposed to barbiturates, so here
metabolic capacity is enhanced indeed (which of course was to be expected). Since 4-dimethylaminophenazone is eliminated almost exclusively by metabolic routes disturbances in renal function will not influence the elimination rate significantly (Leber et al., 1972).

4-methylaminophenazone
No direct data on the pharmacokinetics of this substance are available. Some information, however, may be gathered from investigations on 4-methylaminophenazone—4-methanesulfonate (Novalgin®), since Weiss et al. (1974) provided evidence that this drug already in the gastrointestinal tract decomposes to 4-methylaminophenazone, which then is absorbed. As a matter of fact it is quite unbelievable a priori that a relatively strong and very little lipophilic acid like 4-methylaminophenazone—4-methanesulfonate would be absorbed as such. The 4-methylaminophenazone appears to be readily absorbed and also eliminated at a high rate. From the data of Weiss we calculated a halflife of about 2.5 hrs for 4-methylaminophenazone. Brunk et al. (1974) studied the effect of thyroid status on the metabolism of 4-methylaminophenazone—4-methanesulfonate in man. Since also after i.m. or i.v. administration of 4-methylaminophenazone—4-methanesulfonate the drug itself cannot be found in plasma, but only 4-methylaminophenzone, we may assume that the kinetic data published by these authors should be interpreted as reflections of 4-methylaminophenzone elimination. In normal subjects they found halflives of about 3 hrs, but significantly higher values were measured in hypothyroid patients. Large interindividual variations were observed.

4-isopropylaminophenazone
This drug (trade name Isopyrin®) is used in combination with phenylbutazone in the preparation Tomanol®, which also has been the dosage form administered in the few studies that have been published. 4-isopropylaminophenazone appears to be rapidly taken up in the bloodstream after oral or intramuscular administration. It is distributed over the total watery phase of the body, and only negligibly bound to plasma proteins (Richarz et al., 1959). The drug crosses the placenta barrier readily and is excreted into milk in man (Gensichen et al. 1964). On basis of plasma concentration data in literature (Gensichen et al., 1964; Schmid, 1959) we made rough estimates of the halflife (7–9 hrs) and the volume of distribution (about 50 l) of 4-isopropylaminophenazone. We have, however, serious doubts on the specificity of the analytical procedure applied in these studies, so the estimates should be regarded with due reserve. A last
observation worth mentioning with respect to 4-isopropylaminophenazone is that in rats and guinea-pigs the drug was found to be absorbed also percutaneously (Vogel et al., 1967).

4-aminophenazone
According to Huckabee (1956) 4-aminophenazone is a useful test-substance for determining the volume of total body water, over which it is uniformly distributed. The compound is slightly protein bound. After i.v. infusion Huckabee obtained halflives averaging about 10 hrs and distribution volumes of 0.5 l per kg body-weight. In his study the subjects remained supine but change of posture was noticed to increase the rate of disappearance of 4-aminophenazone. Vesell et al. (1975) determined the halflife of 4-aminophenazone on basis of the urinary excretion of this metabolite after administration of 4-dimethylaminophenazone. The indirect estimates obtained this way varied from 5-20 hrs with an average of 12.6 hrs. All these data, however, are highly disputable, since there is strong evidence, both from the present study and from animal experiments by Sawchuk (1972), that the disposition of 4-aminophenazone is governed by non-linear processes. In the discussion of the results of our study we will analyse these processes in more detail.

The present investigations were planned primarily to establish possible general principles in the elimination of the 4-aminophenazone derivatives. Especially since literature data provide only scarce and indirect information, mostly based on non-specific analytical procedures, it might be expected that a systematic approach as we used, would yield results that are useful and important also from a practical point of view. The drug 4-dimethylaminophenazone which is the only one in the series that has been studied in detail earlier, was mainly included in this study for reference purposes, since in normal subjects it gives an impression of the oxidative dealkylation activity of the liver.

MATERIALS AND METHODS

4-Dimethylaminophenazone was obtained from OPG, Utrecht, The Netherlands; 4-methylaminophenazone from Hoechst, Frankfurt, Germany; 4-aminophenazone from Merck, Darmstadt, Germany and 4-acetylaminophenazone from Aldrich, Milwaukee, Wisconsin, USA. 4-Isopropylaminophenazone (Isopyrin®) was supplied by Byk Nederland, Zwanenburg, The Netherlands, which is gratefully acknowledged. The sub-
stance was the HCl salt and contained 2.5% amylum solani (potato starch), but was used as such. Since all analytical procedures, including calibration graphs for gas chromatography, were performed with the same lot of the compound, no corrections for this 'contaminant' nor for the chloride had to be made at all. The same holds true with regard to 4-methylaminophenazone, which also was used as the HCl salt. The other drugs were used as the free base.

Preparation of the samples and gas chromatographic analysis were performed as described in chapter 2. Chloroform–water distribution coefficients were determined as described in chapter 3.

**Drug administration**

Six male healthy volunteers participated in this study. Every subject received an oral dose of any of the four drugs under investigation, in a random order at biweekly intervals. Some single experiments were repeated 1 year later. The results of these will be discussed separately in comparison with the findings of the main part of the investigations.

The usual precautions with respect to reproducibility of drug absorption were taken: an overnight fast prior to and an additional three hours fast after drug ingestion. At regular intervals after drug intake blood samples were taken from a fore-arm vein, to a total of 10–12. Time and total volume of every urine production were registered and samples of every aliquot were collected for analysis. This was done for a period of at least 30 hrs, depending upon the pharmacokinetic characteristics of the specific drugs.

All drugs were administered in a dose of 600 mg as a solution in 100 ml water. The solutions were freshly prepared, just before ingestion.

**RESULTS AND DISCUSSION**

The complete cross-over design of this study allows a comparison of the pharmacokinetics of each drug within the same individuals. In view of the huge interindividual variations we observed, such a design is an essential prerequisite for comparative purposes. Some examples of the curves obtained are given in figure 10.7. The drawn lines represent the best fit according to the open one compartment model which appears to describe the data very satisfactorily. Only as far as 4-aminophenazone is concerned an exception has to be made to this general statement, as will be shown in part D of this chapter. The pharmacokinetic parameters that were obtained by computer fit are summarized in table 10.5. Similar to the
findings concerning phenazone and 4-isopropylphenazone, also here large interindividual fluctuations are striking.

TABLE 10.5

Pharmacokinetic parameters for 4-aminophenazone derivatives after oral administration (D=600 mg).

<table>
<thead>
<tr>
<th>Subject</th>
<th>V/F (1)</th>
<th>$k_{ce}/F$ (ml/min)</th>
<th>$r_{el}$ (min)</th>
<th>$t_{1/2}$ (min)</th>
<th>$r_a$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JB</td>
<td>(23,63,178)</td>
<td>129</td>
<td>450</td>
<td>284</td>
<td>197</td>
</tr>
<tr>
<td>AD</td>
<td>(23,64,177)</td>
<td>44</td>
<td>150</td>
<td>290</td>
<td>201</td>
</tr>
<tr>
<td>HK</td>
<td>(23,73,181)</td>
<td>112</td>
<td>440</td>
<td>255</td>
<td>177</td>
</tr>
<tr>
<td>PM</td>
<td>(22,90,195)</td>
<td>55</td>
<td>120</td>
<td>461</td>
<td>320</td>
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<tr>
<td>RS</td>
<td>(22,70,190)</td>
<td>124</td>
<td>870</td>
<td>143</td>
<td>99</td>
</tr>
<tr>
<td>RSi</td>
<td>(20,72,183)</td>
<td>127</td>
<td>340</td>
<td>376</td>
<td>261</td>
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</table>

4-METHYLAMINOPHENAZONE

<table>
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<tr>
<th>Subject</th>
<th>V/F</th>
<th>$k_{ce}/F$ (ml/min)</th>
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<th>$t_{1/2}$ (min)</th>
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<td>HK</td>
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<td>310</td>
<td>93</td>
<td>64</td>
<td>6</td>
</tr>
<tr>
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<tr>
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<td>75</td>
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<tr>
<td>RSi</td>
<td>53</td>
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<td>148</td>
<td>102</td>
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4-ISOPROPYLAMINOPHENAZONE

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<tr>
<th>Subject</th>
<th>V/F</th>
<th>$k_{ce}/F$ (ml/min)</th>
<th>$r_{el}$ (min)</th>
<th>$t_{1/2}$ (min)</th>
<th>$r_a$ (min)</th>
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<tr>
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<td>PM</td>
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<td>177</td>
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4-DIMETHYLAMINOPHENAZONE

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<th>$r_{el}$ (min)</th>
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<td>118</td>
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<tr>
<td>AD</td>
<td>46</td>
<td>170</td>
<td>277</td>
<td>192</td>
<td>17</td>
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<tr>
<td>HK*</td>
<td>115</td>
<td>170</td>
<td>277</td>
<td>192</td>
<td>17</td>
</tr>
<tr>
<td>PM*</td>
<td>56</td>
<td>210</td>
<td>267</td>
<td>185</td>
<td>0</td>
</tr>
<tr>
<td>RS</td>
<td>95</td>
<td>530</td>
<td>178</td>
<td>124</td>
<td>9</td>
</tr>
<tr>
<td>RSi*</td>
<td>59</td>
<td>1140</td>
<td>52</td>
<td>36</td>
<td>0</td>
</tr>
</tbody>
</table>

1. Numbers in parentheses represent age (yr), bodyweight (kg) and height (cm); all subjects are male.
   * Plasma curve non-linear, see part D of this chapter.
   □ Could not be determined because of non-linear plasma curve (cf. chapter 13).
Figure 10.7
Plasma curves obtained after oral administration of 4-aminophenazone and 3 derivatives (D = 600 mg) in the same individual.
Absorption

The four drugs are very rapidly absorbed from the aqueous solution administered. This is not unexpected since the drugs combine a reasonable water solubility with a rather high lipid solubility. The absorption appears to start immediately after drug intake as evidenced by the absence of any lag-time in the fitting procedure and by the fact that often the peak concentration in plasma had already been reached before the first blood-sample was taken (10–15 min after administration). In such cases the estimated value of the time constant for absorption cannot be very accurate since no data points are available in the actual absorption phase. This may explain the extremely low time constants for absorption (less than 1 minute) that are obtained in some instances. The error in these estimates is quite high, although it can easily be seen that their upper limit will not exceed some 3 min. On the other hand in this context, the question arises how fast the absorption process maximally can be. The most easy and understandable way of approaching this problem is by regarding absorption as a clearance process in which the gastrointestinal tract is cleared from drug. Obviously this clearance then is limited by the bloodflow around the intestinal system (the bases under consideration will practically not be absorbed from the stomach). The other determinant with respect to the absorption rate is the volume that has to be cleared. It can easily be seen that the shortest time constant for absorption, that may be expected, is given by

\[ \tau_a = \frac{V_o}{k_{Ca, \text{max}}} = \frac{V_o}{f_1} \]  

where  
\( \tau_a \) = time constant for absorption  
\( V_o \) = volume from which absorption occurs  
\( k_{Ca} \) = ‘clearance constant’ for absorption  
\( f_1 \) = intestinal bloodflow

Now the problem is to insert reasonable estimates for \( V_o \) and \( f_1 \) in this equation. Taking into account that the drug solution is ingested after an overnight fast and that the drugs will not precipitate in the stomach, it is likely that the solution passes directly through the stomach to the small intestine. Since also the small intestine, certainly the first part, will be empty, absorption there will start immediately and the effective volume from which absorption takes place will not be much larger than the volume administered. Furthermore, the small intestine is known to be able
to absorb water at a very high rate, which might lead to an even smaller value for $V_0$. On the other hand it is very difficult to estimate the blood-flow in the absorbing region. The small intestine is highly perfused, the blood-flow may be in the order of 1000 ml/min (somewhat below the bloodflow in the portal vein, which is approximately 1200 ml/min). Clearly, however, only a fraction of this total intestinal bloodflow represents the flow in the region where actual drug absorption takes place, since the absorption process will be completed already in the first part of the small intestine. Nevertheless these data indicate that under the experimental conditions of our study the drugs can be absorbed extremely fast. When for instance in equation 10.4 values for $V_0$ and $k_{C_a,max}$ are inserted of 100 ml and 100 ml/min respectively the time constant $\tau_a$ would be 1 min, but it would not be surprising that even smaller values were encountered since the effective $V_0$ might be smaller and the effective $k_{C_a}$ might be higher than the values in this rough estimate.

**Volume of distribution**

The idea that the volume of distribution of pyrazolone derivatives usually is in the order of the volume of total body water seems to be confirmed also by the data in table 10.5. Of course it should be stressed again that since the drugs were administered orally, the volume terms are only determined relatively to the biological availability, which is essentially unknown. Now the high rate and instantaneous start of absorption might suggest that the availability of the drugs from the solution is practically complete. In many cases this suggestion is supported indeed by the fact that the $V/F$ estimates correspond to half a liter per kg body-weight or less. There are, however, some clear exceptions, especially in case of 4-methylaminophenazone where much larger volumes are found. Nevertheless also this compound appears to be distributed similarly to the other pyrazolones studied as shown by the quite normal distribution volume exhibited by the subjects PM and AD. Therefore we tend to ascribe the large volume estimates to incomplete biological availability. Also the values found for the clearance constant provide evidence in favour of this assumption, because they are large when the volume is large and because they also have only relative meaning (with respect to $F$). Another possible explanation could be sought in the occurrence of a first-pass effect. Taking subject RS as an example it can easily be derived that when he would have absorbed the whole dose of 4-methylaminophenazone his pharmacokinetic characteristic would give rise to a substantial first-pass effect. The fraction reaching the circulation intact can be formulated as:
which would yield corrected values for \( V \) and \( k_{\text{Cel}} \) of 78 l and 550 ml/min respectively. Despite this first-pass correction, still an incomplete bioavailability would have to be assumed. Furthermore, it should be noticed that the first-pass assumption for the other subjects has much less significant influence. Therefore it is an unlikely explanation, especially since all estimates can easily be brought to very acceptable values by assuming that the subjects RS, JB, R Si and HK have absorbed 4-methylaminophenazone only for about 40%. It should be noticed, however, that in our experimental set up a strongly impaired absorption can hardly be reconciled with the very high absorption rates observed. This discrepancy seems to point at intestinal metabolism as a possible process contributing to the apparently incomplete absorption. It is well-known that the intestinal flora exhibits a variety of drug metabolizing properties (Scheline, 1968; Goldman, 1973). In view of the work of Weiss et al. (1974) it is plausible that also alkylated 4-aminophenazone derivatives are subject to such metabolism. Furthermore, the epithelial cells of the intestinal mucosa are equipped with several drug metabolizing enzyme systems so that biotransformation may take place during absorption (see for a review of these complications before and during drug absorption: Riegelman and Rowland, 1973). The same discussion can be applied to some of the 4-dimethylaminophenazone data (especially subject HK) and to some of the 4-aminophenazone experiments (especially RS and HK). This last drug, however, exhibits a very peculiar pharmacokinetic pattern as will be discussed in detail below.

In general the distribution of a drug is governed by its physicochemical properties: \( pK_a \), water solubility, lipid solubility, protein binding etc. Apart from these factors a specific interaction with certain tissue components may become quantitatively so important that it influences overall distribution appreciably. For the phenazone derivatives, however, there are no indications at all for such a specific tissue binding. Only the general physicochemical factors remain. The \( pK_a \) is important in so far as it can cause differences in the fraction of drug that is unionized and therefore freely diffusible through biomembranes. 4-Dimethylaminophenazone has a \( pK_a \) of 5, 4-aminophenazone of 4.1, the other two derivatives presumably will have a \( pK_a \) between these two extremes, although the values are not exactly known. For the degree of ionization this means that at physiological pH (7.4) all 4 drugs are practically fully unionized (more than 99.7%). Also with regard to solubility in aqueous and organic phases the
four drugs differ only to a minor extent. Measurements of the partitioning behaviour indicate that the chloroform–water partition coefficients at pH 7.4 decrease in the following order: dimethylamino > amino > isopropylamino > methylamino. At first sight this is a somewhat unexpected sequence since one would think that lipid solubility increases and water solubility decreases with increasing bulk of the substituent. Probably more subtle changes in electron distribution upon substitution at the nitrogen atom predominate. However, without looking for sophisticated arguments, it is justified to note that solubility factors hardly can influence the distribution pattern, since the difference in partition coefficients is at most a factor 2 (cf. chapter 3). Also the partition ratio between saline and erythrocytes in vitro seems to rule out significant influence on drug distribution. For every one of the 4 compounds this ratio was near to one, interindividual differences being hardly significant. A factor that can have important consequences for the apparent volume of distribution as estimated on basis of total plasma concentrations is the possible binding to plasma proteins. However, phenazone derivatives of the type we are dealing with are only slightly protein bound (Brodie et al., 1950a and 1950b). From preliminary data we assume the upper limit of the percentage protein binding to be 15, at plasma concentrations of about 10 mg/l. Assuming for the rest a homogeneous distribution over plasmawater and cellwater, it can be derived that the distribution volume on basis of the plasma concentration may be some 10% lower than the total body fluid. Some of the estimates for V/F in table 10.5 indeed seem to be slightly below the volume of the total body fluid, so it appears worthwhile to consider the possible influence of protein binding in more detail. More direct measurements will be needed to clarify this matter.

Elimination
The individual values for the time constant for elimination $\tau_{el}$ that are given in table 10.5 show a large interindividual variation. The most striking variation occurs with 4-aminophenazone and 4-methylaminophenazone. This is also reflected in table 10.6 where the mean time constants together with their standard deviation are summarized for each of the four drugs. It is interesting to note that due to the large standard deviation the average elimination time constant for 4-aminophenazone is not significantly different from any of the other ones, whereas these three values are all significantly different from each other. Furthermore, the mean time constants for elimination of 4-dimethylamino-, 4-methylamino- and 4-isopropylaminophenazone in each individual appear to be correlated, the level of
**TABLE 10.6**

Average values of $\tau_{el}$ for 4-aminophenazone derivatives

<table>
<thead>
<tr>
<th></th>
<th>Average $\tau_{el}$ ± SD (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-aminophenazone</td>
<td>166 ± 92*</td>
</tr>
<tr>
<td>4-methylaminophenazone</td>
<td>302 ± 108*</td>
</tr>
<tr>
<td>4-isopropylaminophenazone</td>
<td>110 ± 27*</td>
</tr>
<tr>
<td>4-dimethylaminophenazone</td>
<td>155 ± 49*</td>
</tr>
</tbody>
</table>

* significantly different from each other, $P < 0.02$
* not significantly different from any of the other averages, $P > 0.20$

**Figure 10.8**

*Correlation between the time constants for elimination of 4-methylaminophenazone, 4-dimethylaminophenazone and 4-isopropylaminophenazone. The drawn line is obtained by the regression analysis. Since the intercepts are not significantly different from zero also a regression line through the origin is calculated (dotted).*
significance of this correlation being at least $P < 0.05$ (figure 10.8). Again
4-aminophenazone takes a separate position in that its elimination time
constants are not correlated at all with any of the other three ($P > 0.30$).
Although it is no indisputable proof, these correlations suggest a common
elimination mechanism for the three alkylated 4-aminophenazone
derivatives, while 4-aminophenazone itself is eliminated via another route.
Interestingly this is totally in line with that part of the metabolism that is
known: the N-alkyl derivatives are subject to oxidative dealkylation, but
4-aminophenazone is for a major part acetylated and for a minor part
excreted in urine unchanged. Taking all considerations together there
seems to be no reason at all for discussing 4-aminophenazone kinetics in
the context of the pharmacokinetic behaviour of its N-alkylated derivat­
tives. There are even more arguments to discuss 4-aminophenazone
separately, especially the fact that its pharmacokinetic behaviour is clearly
non-linear. As far as possible the time constants for elimination of
4-aminophenazone given in table 10.5 are the real ones, based on the
ultimate steepest and linear part of the plasma concentration-time curve.
Unfortunately it cannot be excluded that some of the estimates are still
too high, because possibly the plasma concentration was not always
followed long enough. Also for this reason we prefer to restrict the
pharmacokinetic comparison to the alkylated derivatives and to pay
special attention to 4-aminophenazone at the end of this chapter.

The rate of elimination of the 4-alkylaminophenazone compounds
decreases in the following order: 4-isopropylamino $>$ 4-dimethylamino $>$
4-methylamino. Referring to the presumed common metabolic pathway,
this sequence might be explained on basis of the ability of the alkyl groups
to resist oxidative metabolic attack. Then the isopropyl group seems to be
very labile and more easily removed than a methyl group, whereas the
presence of a second methyl group on the same nitrogen atom leads to
increased rate of demethylation. One might even think of the number of
carbon atoms attached to the nitrogen, as determinant for the rate of
oxidative dealkylation, the rate being highest in case of 3 and lowest in
case of one carbon atom. It should be remembered, however, that one
cannot be sure that the major part of the metabolism of 4-aminophena­
ze derivatives proceeds via dealkylation since usually not more than
about 50% for the corresponding metabolites could be traced in urine.
Nevertheless it is interesting that the sequence found is the same as that of
renal excretion halflives of the dextro-isomers of the corresponding
N-alkyl-amphetamines in man, which were reported by Vree (1973).
Renal excretion
Alkylated aminophenazone derivatives are excreted in urine unchanged only to a very low extent. Table 10.7 shows the cumulative amounts of intact drug that appeared in urine and fig. 10.9 gives a representative example of the renal excretion rates and cumulative renal excretion as a function of the time after administration. For the sake of completeness also 4-aminophenazone is included, but as stated before these data will be discussed separately at the end of this chapter.

TABLE 10.7
Cumulative renal excretion (% of dose) of 4-aminophenazone derivatives

<table>
<thead>
<tr>
<th>Subject</th>
<th>MAP</th>
<th>IAP</th>
<th>DAP</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>JB</td>
<td>2.2 (3.5) 1</td>
<td>0.08</td>
<td>0.32</td>
<td>16.5</td>
</tr>
<tr>
<td>AD</td>
<td>4.9 (7.4) 1</td>
<td>0.65</td>
<td>*</td>
<td>24.6</td>
</tr>
<tr>
<td>HK</td>
<td>-</td>
<td>0.94</td>
<td>0.01</td>
<td>11.3</td>
</tr>
<tr>
<td>PM</td>
<td>6.1 (8.2) 1</td>
<td>0.16</td>
<td>*</td>
<td>41.5</td>
</tr>
<tr>
<td>RS</td>
<td>-</td>
<td>0.28</td>
<td>*</td>
<td>29.6</td>
</tr>
<tr>
<td>RSi</td>
<td>-</td>
<td>0.17</td>
<td>0.35</td>
<td>3.8</td>
</tr>
</tbody>
</table>

1. Percentage excreted as metabolite (4-aminophenazone)
* not detectable
- not determined

MAP = 4-methylaminophenazone
IAP = 4-isopropylaminophenazone
DAP = 4-dimethylaminophenazone
AP = 4-aminophenazone

The renal clearance constant of 4-dimethylaminophenazone and 4-isopropylaminophenazone is equal to or somewhat smaller than the urine-flow, about 1 ml/min. It can safely be assumed that the glomerular filtration rate of these drugs is much higher. Even when protein binding is taken into account the filtration rate will be at least some 75 ml/min. Therefore substantial reabsorption must occur, at a rate higher than or equal to the rate of water reabsorption. This is quite conceivable in the light of the intermediate lipid- and watersolubility of the drugs. For 4-methylaminophenazone the renal clearance constant is somewhat higher than the rate of urine production. Interestingly this drug has the lowest partition coefficient of all phenazone derivatives we studied, which might cause the lowest reabsorption. For some of the volunteers also the amount of
4-aminophenazone after administration of 4-methylaminophenazone was quantified. This amounted up to 8% and although not quantitatively determined, appreciable amounts of 4-aminophenazone appeared in urine after administration of the other derivatives as well. Furthermore, in any case large quantities of 4-acetylaminophenazone were excreted in urine. Unfortunately our gaschromatographic analysis did not allow accurate measurements of the amounts, but rough estimates suggest that especially for 4-methylaminophenazone a major part of the dose administered may be accounted for by renal excretion of acetylated aminophenazone.

Figure 10.9
Renal excretion rate and cumulative renal excretion after oral administration of 4-aminophenazone and derivatives to the same volunteer (D = 600 mg). After administration of 4-methylaminophenazone also 4-aminophenazone is found in urine.

a. Renal excretion of unchanged and demethylated 4-methylaminophenazone.
b. Renal excretion of unchanged 4-isopropylaminophenazone (left) and 4-dimethylaminophenazone (right).
c. Renal excretion of unchanged 4-aminophenazone.
renal excretion rate
(\(\mu g/min, \text{log scale}\))

- 4 isopropylaminophenazone
  - 600 mg oral
  - Subj J &

- 4 dimethylanilinophenazone
  - 600 mg oral
  - Subj J B

cumul renal excretion
(\% of dose)

- 4 aminophenazone
  - 600 mg oral
  - Subject J B

Renal excretion rate
(\(\mu g/min, \text{log scale}\))

cumul renal excretion
(\% of dose)

Renal excretion rate
(\(\mu g/min, \text{log scale}\))

cumul renal excretion
(\% of dose)
Clearance
Considering the factors discussed above in connection with the volume of distribution (and the biological availability) and regarding the data in table 10.5 it seems reasonable to value the clearance constants for 4-isopropylamino-, 4-dimethylamino- and 4-methylaminophenazone at approximately 350, 250 and 150 ml/min respectively. For all practical purposes the clearance is determined by metabolic processes. Speculating once again on a common mechanism, it may be interesting to define the metabolic clearance constant in more detail. As will be shown in chapter 13 a metabolic clearance constant $k_{Cm}$ represents the ratio $\dot{Q}_m/K_M$, where $\dot{Q}_m$ is the capacity of the system involved (e.g. in $\mu$g/min) and $K_M$ is the Michaelis-Menten constant for the enzyme-substrate complex (e.g. in mg/l). When more than one enzymatic pathway occurs simultaneously, the metabolic clearance constant obviously can be given by:

$$k_{Cm} = \sum_{i=1}^{n} \frac{\dot{Q}_{m,i}}{K_{M,i}}$$

(10.5)

This relationship indicates that differences in overall clearance constant can be attributed to differences in metabolic capacity or in the Michaelis-Menten constant. In vitro investigations may shed light upon the question which enzymatic parameter primarily changes upon variation in the substrate exposed. In case of the metabolism of N-alkyl amphetamines by rat liver microsomal preparations there are strong indications that the $V_{max}$ value varies significantly with varying alkyl-group (Henderson et al., 1974), whereas the $K_M$ values change much less (Henderson, 1976). On the other hand there is little doubt that the enzyme system involved exhibits a remarkable multiplicity in the sense that it contains several more or less different enzymes specifically directed towards the individual substrates of an apparently homogeneous group. The correlation we found between the time constants for elimination should be based on a similar correlation in the respective clearance constants and seems to suggest a common denominator for the possibly different enzymes involved. Such a 'unity in multiplicity' could be expected when all enzymatic activities we are dealing with would be reflections of one genetically determined metabolic system. Although this may be an attractive and likely assumption much more work will be needed to confirm it.

One more remark should be made concerning the metabolic clearance of
these drugs. As has been discussed before one may expect a measurable first-pass effect to occur for drugs with a metabolic clearance constant that is not small with respect to the hepatic bloodflow. Since the alkylated aminophenazones have equal and rapidly exchangeable concentrations in plasma and bloodcells the total blood clearance has the same magnitude as the plasma clearance and these are the values that have to be compared with hepatic bloodflow, which normally lies in the range of 1500–2000 ml/min. Clearly, the largest first-pass effect (if any) should be expected to occur in case of 4-isopropylaminophenazone with a metabolic clearance constant of about 350 ml/min. It can easily be calculated that the fraction of the dose metabolized at its first pass through the liver will not exceed some 20%. This implies that the volume of distribution and the clearance constant may be overestimated to the amount of 20% maximally. Mostly, however, the influence of first-pass metabolism will be significantly smaller and will remain within the limits of accuracy of the estimated and computer fitted parameter values. Therefore it seems justified to neglect corrections for first-pass effects with regard to the results of this study.

D. 4-AMINOPHENAZONE ELIMINATION

The drug 4-aminophenazone is eliminated in a way totally different from its N-alkylated derivatives. As pointed out already in the introduction the major metabolic pathway here is N-acetylation. Further substantial quantities are excreted in urine unchanged. In this study large interindividual variations in the rate of renal excretion were observed. Interestingly in all volunteers some evidence was obtained in favour of non-linear renal excretion processes. Fig. 10.10 gives an example of this, from which it is obvious that the rate of excretion in urine is not simply proportional to the average plasma concentration. Unfortunately excretion of drug seemed to be strongly dependent on the rate of urine production. Although it was impossible to find a correlation between the amount excreted unchanged and the average urine flow in the group of subjects, the individual fluctuations in the renal excretion rate over a certain period were parallel to fluctuations in the amount of urine produced in that period. The lack of correlation between the two variables in an overall picture most probably is due to non-linearities in the excretion mechanism as such. Assuming that the renal excretion rate can be represented by a combination of glomerular filtration and tubular secretion, we were able to derive the relevant parameters in 4 of the subjects. These are summarized in
Renal excretion rate of 4-aminophenazone as a function of the corresponding average plasma concentration in two volunteers. Note the non-linear curves, indicating a possible occurrence of tubular secretion.

**TABLE 10.8**

Parameters describing tubular secretion of 4-aminophenazone

<table>
<thead>
<tr>
<th>Subject</th>
<th>$T_M$ (μg/min)</th>
<th>$K_T$ (mg/l)</th>
<th>Filtration (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>60</td>
<td>1.4</td>
<td>35</td>
</tr>
<tr>
<td>PM</td>
<td>120</td>
<td>1.4</td>
<td>35</td>
</tr>
<tr>
<td>RS</td>
<td>140</td>
<td>0.4</td>
<td>90</td>
</tr>
<tr>
<td>RS₁</td>
<td>15</td>
<td>0.5</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 10.8, but they should be regarded with due reserve since the relative error in these estimates may be as high as 100%. Nevertheless it appears that the secretory pathway can easily be saturated, that its transport maximum is in the order of 100 μg/min (6 mg/hr) but highly variable inter-individually and that glomerular filtration (including back diffusion) causes a clearance which is usually below the maximal value attainable but definitely higher than the rate of urine production. The excretion of unchanged 4-aminophenazone is completed within 25 hrs after drug intake.
and the amount is highly variable (4–40% of dose, see table). Large amounts of 4-acetylamino-phenazone appear in urine as well. This metabolite is excreted over a much longer period. Unfortunately our analytical procedure did not allow an accurate measurement of the acetyl-derivative, but according to rough estimates in some subjects it seems to account for the missing part of the total dose administered. In chapter 13 it will be demonstrated that a mechanism of elimination which is saturable at the prevailing plasma concentrations can lead to deformations in the plasma concentration curve, provided that its clearance constant under unsaturated conditions (i.e. its maximum value) represents more than 20% of the total body clearance. The deformations will only be clearly visible when this percentage becomes about 50. The maximal clearance constant that can be obtained by the secretory mechanism can be defined as the ratio $T_M/K_T$ and then it can easily be derived that in some of the subjects certainly a non-linear plasma curve may result from the tubular secretion process. This is found indeed in several experiments. Intriguingly, however, the plasma curve of for instance subject RS did not show any indication at all for non-linear processes (see fig. 10.12), whereas his secretion characteristics might be expected to lead inevitably to a non-linear plasma curve. On the other hand the plasma curve of subject RSi obviously can only be described by non-linear processes (figure 10.11), although in this person renal clearance gives no reason at all to assume visible deviations from first-order behaviour. Now the first example (RS) could be explained by assuming that the plasma curve was not followed long enough to observe the predicted bending of the curve. This is a likely possibility since the estimated $K_T$ is rather low ($K_T = 0.4 \text{ mg/l}$) and since the bending occurs at plasma concentrations in the order of magnitude of $K_T$ and the ultimate straight line corresponding to maximally attainable clearance is only reached when the plasma concentration is still much lower, e.g. $< 0.1 K_T$ (chapter 13). As a matter of fact this implies that the slope of the curve for RS will be concentration-dependent, since it represents the elimination at more or less saturating levels. It will be shown below that the concentration-dependence of the half-life was observed indeed. So far the argumentation is satisfactory, but difficulties remain with regard to the second example (subject RSi) where a clearly non-linear plasma curve is found contrary to what might be expected on basis of the low renal clearance involved. These observations seem to point out that 4-aminophenazone elimination is more complicated than a combination of just one saturable mechanism (tubular secretion) with a linear elimination system consisting of an excretory and a metabolic pathway. This is substantiated by the
discrepancy prevailing between the characteristic parameters of the non-linear process derived from analysis of renal excretion, and those derived from appropriate analysis of the non-linear plasma curves. This last type of analysis will be discussed in detail in chapter 13. The kinetic and metabolic parameters that could be calculated by thorough analysis of the plasma curves after 4-aminophenazone are given in table 10.9. Three of the volunteers exhibited no signs of non-linear pharmacokinetics, the other three did. Two of the volunteers received the same drug in the same dose one year later. Also the parameters derived from this repeated experiment are inserted in table 10.9. Of course it is quite an unusual phenomenon that the same drug is eliminated according to two different models in different subjects, who all are in a good physical shape. The fact that the drug under consideration is metabolized by acetylation may be specifically meaningful in this respect. It is well-known that the rate at which people are able to acetylate some drugs is not normally distributed, but that a
TABLE 10.9

Parameters for linear and non-linear elimination of 4-aminophenazone. (see text for further explanation).

<table>
<thead>
<tr>
<th>Subject</th>
<th>V/F (l)</th>
<th>k_Ce/F (ml/min)</th>
<th>τ_el (min)</th>
<th>K_M (mg/1)</th>
<th>f</th>
<th>Q_m (mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JB</td>
<td>41</td>
<td>350</td>
<td>118</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>46</td>
<td>170</td>
<td>277</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HK</td>
<td>5.74</td>
<td>115</td>
<td>1120</td>
<td>103</td>
<td>3.5</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>4.75</td>
<td>51</td>
<td>520</td>
<td>98</td>
<td>4.1</td>
<td>0.77</td>
</tr>
<tr>
<td>PM</td>
<td>56</td>
<td>210</td>
<td>267</td>
<td>4.3</td>
<td>0.64</td>
<td>0.57</td>
</tr>
<tr>
<td>RS</td>
<td>5.74</td>
<td>95</td>
<td>530</td>
<td>178</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.75</td>
<td>44</td>
<td>160</td>
<td>277</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSi</td>
<td>59</td>
<td>1140</td>
<td>52</td>
<td>2.2</td>
<td>0.93</td>
<td>2.33</td>
</tr>
</tbody>
</table>

Genetically determined bimodal distribution occurs. The two groups are referred to as slow and fast acetylators. The best-known example is the antituberculous drug isoniazid (Bönicke and Reif, 1953; Bönicke and Lisboa, 1957; Evans et al., 1960). According to Peters et al. (1965) the slow-acetylation phenomenon is caused by a genetically determined reduced liver acetyltransferase activity. Several other drugs exhibit the acetylation polymorphism as well, but there are also some drugs that appear in urine in acetylated form but that do not show polymorphism (Evans and White, 1964). Presumably there are several acetylation enzymes present, not only in the liver but also elsewhere in the body (Hearse and Weber, 1973). Substantial acetylation activity is found for instance in blood cells. Interestingly the acetyltransferase present in these cells is apparently not of the polymorphic type (Drayer et al., 1974). The blood contains also deacetylation activity but the rate of deacetylation is much smaller than that of acetylation.
Returning to the elimination of 4-aminophenazone one is forced to assume that the mechanism of this elimination will be very complex. On the one side there is the renal excretion, partly linear, partly definitely non-linear, with probably large interindividual variation in determining parameters. On the other side we are dealing with metabolic transformation, for the major part acetylation in which a lot of different enzymes may be involved. The discrepancy between the renal excretion parameters and the parameters describing the plasma decay curve suggest that also the acetylation may be a non-linear process, where at 'therapeutic' dose levels saturation phenomena can become predominant. Unfortunately it is very difficult to establish on the basis of plasma curves whether one or more non-linear mechanisms of drug elimination are prevailing. As will be shown in chapter 14 it is virtually impossible to discriminate between one or more partly saturated mechanisms since the pooling of several non-linear pathways usually leads to plasma curves that can easily be described by just one non-linear clearance function. Of course the parameters estimated on basis of such a single non-linear clearance function are basically dose-dependent, so that they can hardly be used for pharmacokinetic modelling. They have only significance as operational parameters within a relatively small dose range. Now accepting the fact that we have some evidence that two non-linear mechanisms are involved in 4-aminophenazone elimination together with at least one linear, we have to content ourselves with the following definition of the clearance function.

\[
V_{Cel} = \left[ 1 - f + \frac{fK_M}{K_M + C} \right] k_{Cel} \tag{10.6}
\]

where

- \( V_{Cel} \) = the plasma-clearance function
- \( K_M \) = the apparent Michaelis-Menten constant between drug and elimination mechanism; in case of more than one non-linear pathway, it is some kind of weighted average of the individual values
- \( k_{Cel} \) = the maximally attainable clearance constant. This clearance is obtained when no saturation at all occurs \((C \ll K_M)\)
- \( C \) = plasma concentration of drug
- \( f \) = that part of the maximal clearance \( k_{Cel} \) that proceeds via potentially saturated pathways

(details concerning this equation and its derivation and implications can be found in chapter 13).
Equation 10.6 indicates that the clearance will always be smaller than $k_{Ce}$, unless $C << K_M$. As a matter of fact, with decreasing plasma concentration the clearance is increasing and the elimination time constant (so the half-life as well) is shortening. This effect results in plasma curves as exemplified in figure 10.11. Although theoretically bending of the semi-logarithmic plasma curve must occur, provided that $f$ is large enough, one can imagine that it will not always be detected in an experimental situation. It can easily be overlooked when the plasma concentration is not followed to levels smaller than $K_M$. This might be the case of the three subjects in this study, who did not show any signs of non-linear elimination. From equation 10.6 it can be derived that in such a case an apparent halflife ($t_{1/2}^{app}$) should be expected, that is defined by:

$$t_{1/2}^{app} = (\ln 2) \tau_{el, app} = 0.693 \frac{V}{k_{Ce}} \frac{K_M + C}{K_M + (1-f) C}$$

where $V$ = the volume of distribution of the drug (and the other symbols as defined above)

This equation implies that the apparent halflife can have values all over the range from $0.693V / k_{Ce} (1-f)$ to $0.693V / k_{Ce}$ and that the value can be expected to be higher, the higher the plasma concentration range is in which the measured data points are lying. One of the volunteers with a seemingly first-order elimination received an oral dose of 600 mg 4-aminophenazone twice with an interval of approximately 1 year. At these two occasions very different plasma levels were measured and the apparent halflife was 1.5 times higher at the time that the highest plasma levels were measured (192 versus 124 minutes; see figure 10.12). This difference is in line with the predictions from equation 10.7. Furthermore it was interesting to observe that on both occasions the same amount of drug was excreted unchanged, although in the experiment with the low plasma levels most probably only about half as much of the dose had reached the general circulation as compared with the other experiment. This observation again is compatible with a clearance function as given in equation 10.6 since a saturable excretion mechanism yields a relatively higher contribution to overall elimination when the plasma levels of the substance to be eliminated are lower. One has to be careful, however, in his conclusions since the two experiments are separated by a long time-interval, in which the elimination characteristics might have changed. On the other hand individual acetyl-
Figure 10.12

Plasma curves after oral administration of 4-aminophenazone (600 mg) to the same volunteer on two different occasions (interval about one year). Note the large differences in half-life and apparent volume of distribution. See text for further explanation.

Elimination rates have been reported to remain strikingly constant over periods of at least a year (Das and Eastwood, 1975; White and Evans, 1968).

At this stage discussion of the results of the other repeated experiment is pertinent. Subject HK received his doses at exactly the same time as RS. Contrary to RS, however, his plasma curves showed clear signs of nonlinear kinetics (see figure 10.13). It is interesting that he also had much lower plasma levels in the first than in the second experiment. But, what is more, the ultimate half-life was practically identical at both occasions (103 and 98 minutes). Also the estimates of $K_M$ and $f$ were not significantly different in the two experiments. The relative constantness of the parameters estimated for HK together with the literature data mentioned suggest that our explanation of the plasma curves of subject RS will be
Plasma curves after oral administration of 4-aminophenazone (600 mg) to the same volunteer on two different occasions. The elimination is clearly non-linear and there is a striking difference in the apparent volume of distribution. Nevertheless, the other pharmacokinetic parameters as obtained by computer fit are about the same for both curves. See text for further explanation.

It remains a problem how the large differences in the amount of drug that reaches the circulation intact arise. Analogously to the alkylated 4-aminophenazone derivatives, impaired absorption is not a likely cause. Metabolism in the intestinal contents or in the gut wall is much more probable, but it is not clear how this could be so variable (even intra-individually). A further possibility might be the occurrence of a hepatic first-pass effect. Some of the estimates for $k_{\text{Cel}}/F$ are in the order of one third or more of the hepatic blood flow, which might give rise to substantial metabolism at first pass through the liver, provided that a major part of this clearance is a consequence of biotransformation in the liver. However, since the contribution of renal excretion certainly is not negligible and since it is possible that more than one acetyulating system (also extra-
hepatic) takes care of biotransformation, the significance of a first-pass effect is doubtful. This is the more so, when a capacity-limited hepatic metabolism occurs. In that case the metabolic system may become saturated during the passage of large amounts of drug from the portal vein through the liver to the general circulation. Figure 10.14 shows a theoretical example of such a saturable first-pass effect and it is obvious that only very minor amounts of drug are extracted at first-pass, although the ultimate clearance constant is high. Especially since in case of 4-amino-phenazone $K_M$ presumably is very small, the initial concentrations in the portal vein can easily saturate the hepatic metabolic system. It should be mentioned that Das and Eastwood (1975) found the acetylation of sulfapyridine to be independent of the dose (2 to 8 g per day), which result, however, cannot be extrapolated to other drugs that are largely acetylated. Whether or not a so-called capacity-limited elimination becomes detectable is not dependent on the metabolic capacity but only on the Michaelis-Menten constant. This $K_M$ may differ to a huge extent among various drugs.

![Figure 10.14](image_url)

**Figure 10.14**  
Theoretical curves for drugs with capacity-limited elimination, that have a high maximal clearance constant. Despite a very high metabolic clearance usually no substantial first-pass effect is observed, since the metabolism is easily saturated. The parameters used for the simulation are: $V_G = V_L = 5 \text{l}$, $V_C = 35 \text{l}$, $k_{Cl} = 2 \text{l/min}$, $k_{LC} = k_{CL} = 1.5 \text{l/min}$, $k_{Cr} = 0.1 \text{l/min}$ and further the combinations of $K_M = 5 \text{mg/l}$ with $Q_m = 5; 3.75$ and $2.5 \text{mg/min}$ (curves 1, 2 and 3) and of $K_M = 2 \text{mg/l}$ with $Q_m = 2$ and $1 \text{mg/min}$ (curves 4 and 5).
TABLE 10.10

Percentage of 4-aminophenazone excreted unchanged with $\tau_{el}$ and $f$.
(see text for further explanation)

<table>
<thead>
<tr>
<th>Subject</th>
<th>$Q_r%$</th>
<th>$\tau_{el}$ (min)</th>
<th>$f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>JB</td>
<td>16.5</td>
<td>118</td>
<td>-</td>
</tr>
<tr>
<td>AD</td>
<td>24.6</td>
<td>277</td>
<td>-</td>
</tr>
<tr>
<td>HK</td>
<td>5.74</td>
<td>11.3</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>4.75</td>
<td>103</td>
<td>0.77</td>
</tr>
<tr>
<td>PM</td>
<td>41.5</td>
<td>267</td>
<td>0.64</td>
</tr>
<tr>
<td>RS</td>
<td>5.74</td>
<td>29.6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4.75</td>
<td>28.5</td>
<td>-</td>
</tr>
<tr>
<td>RSi</td>
<td>3.8</td>
<td>52</td>
<td>0.93</td>
</tr>
</tbody>
</table>

When comparing the amount of 4-aminophenazone excreted unchanged with the time constants for elimination, one observes some kind of relationship (table 10.10). It seems that the subjects who have the longest time constant for elimination excrete the largest amounts unchanged in urine. Most probably, this means that in these cases the acetylation is slower, indicating that the rate of elimination is a good reflection of the rate of metabolic transformation. As an example especially comparison of the data of the subjects PM and RSi is very illustrative. It is also interesting that the fraction $f$ in these subjects shows an inverse relationship with the percentage excreted unchanged. This suggests even that the major part of the fraction that is eliminated by potentially saturated systems is based on metabolic routes. So, despite of the sometimes substantial renal excretion, the profile of the plasma curve appears to be determined primarily by biotransformation (acetylation) of the drug. When we examine the time constants for elimination in this respect we tend to conclude that also in 4-aminophenazone elimination a distinction between slow and fast acetylators may be made. In this sense the subjects RSi, JB and HK might be classified as fast, the other three as slow acetylators. However, this division is quite arbitrary, since another division may be necessary when other doses of the drug are applied. The metabolic clearance and therefore the elimination rate of the drug is determined by both the capacity of the

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metabolic system and the apparent Michaelis-Menten constant. Differences in capacity can be the result of differences in the amounts of enzyme and differences in the nature of the enzyme. The Michaelis-Menten constant probably will be solely determined by the nature of the enzymes involved. When we compare again the subjects PM and RSi it is obvious that $K_M$ is different but the capacity as well (the capacity is equal to $fK_Mk_{cel}$, so 0.57 mg/min and 2.33 mg/min for PM and RSi respectively). Taking into account the relative errors of the estimates one may conclude that the difference in capacity is much more significant than the difference in $K_M$. At the moment further investigations into this matter are planned in order to obtain more straightforward data. It is remarkable, however, that the present results point to differences in metabolic capacity as the predominant cause of variations in the rate of acetylation of 4-amino-phenazone and that this co-incides with the results of Peters et al. (1965) on isoniazid acetylation.

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CHAPTER 11 PARACETAMOL AND PHENACETIN

INTRODUCTION

At the end of the 19th century acetanilide was introduced as an antipyretic agent. Acetanilide is a rather toxic compound, but it was soon established that the pharmacological effect probably resides in a metabolite: 4 acetylaminoephanol (paracetamol). Already at the turn of the century paracetamol and its ethoxy-derivative phenacetin became available for antipyretic-analgesic purposes. The use of phenacetin, especially in combination preparations became widespread. Probably the fact that phenacetin has some psychotropic effect, causing a light euphoria, and leading to habituation, has contributed to its popularity. Abuse of phenacetin-containing analgesic preparations is not seldom encountered and is very dangerous in view of the severe nephrotoxicity of such preparations on long-term use (Raaflaub and Dubach, 1972; Höffler et al., 1973; Rüegger et al., 1973; Bock et al., 1973; Dubach et al., 1974; Dubach et al., 1975).

Although it is still a point of controversy whether phenacetin or acetylsalicylic acid (with which it is usually combined) is the most nephrotoxic drug (e.g. Presscott, 1972), it seems that withdrawal of phenacetin from some proprietary analgesics has a beneficial effect on the incidence and seriousness of analgesic nephropathy (Murray, 1972). From the recognition of the risks of phenacetin, a tendency originated to replace it by paracetamol which never had been as popular as phenacetin and which in most countries was not marketed until the fifties. Accordingly the experience with this drug is limited as compared with phenacetin, but up to now no serious side-effects of chronic administration of normal doses have been reported. Paracetamol overdose may cause a lifethreatening hepatic damage (Presscott et al., 1971; Prescott and Wright, 1973; Clark et al., 1973; James et al., 1975; Mitchell et al., 1973a, b; Jollow et al., 1973; Potter et al., 1973).

Glutathione has a protective effect against the hepatic necrosis and on rational grounds glutathione-like nucleophiles, such as cysteamine, have been proposed and successfully applied to the treatment of severe paracetamol overdosage (Mitchell et al., 1974; Prescott et al., 1974). Possibly methionine and vitamin E (α-tocopherol) provide a similar protection (McLean and Day, 1975; Kelleher et al., 1974). The doses at which this serious toxicity appears can hardly be estimated, but it seems that
10 grams can be fatal, although much higher doses have been tolerated without irreversible damage.

In normal doses however paracetamol is one of the safest minor analgesics, which certainly deserves a place in analgesic therapy. As far as phenacetin is concerned the situation is different: it will be difficult to find any argument at all for retaining this drug on the market.

PHARMACOKINETICS

a. Phenacetin
Plasma levels of phenacetin after oral administration have often been reported (Brodie and Axelrod, 1949; Prescott et al., 1968; Prescott, 1969; Prescott et al., 1970; Harris and Riegelman, 1969; Kampffmeyer, 1971; Pantuck et al., 1972). The dosages applied usually were high (1.5 g) in order to obtain easily measurable plasma levels. Half-lives of 0.5—1.5 hrs have been measured. On basis of the literature data we estimated the volume of distribution to be at least 60 l.

Methods
Since phenacetin as a rule is used in low dosage (mainly in combination preparations where the phenacetin content seldom exceeds 250 mg) we administered 500 mg of phenacetin to 8 fasting, healthy volunteers, 6 male, 2 female, age of 19—23 years, body weight 55—74 kg. Phenacetin was emulsified with the use of Tween 80. Blood samples were taken from a forearm vein at regular intervals after drug intake for a total period of 8 hrs. Urine samples were collected up to 24 hrs after drug administration. Gas chromatographic analysis was performed as described chapter 2.

Results and discussion
In 6 volunteers the phenacetin level in plasma did not exceed 1 mg/l, the 2 remaining subjects showed maximum levels of 3—4 mg/l. The profile of the ‘plasmacurve’ was very irregular and not useful for pharmacokinetic analysis. Urinary excretion of unchanged phenacetin accounted for less than 0.5% of the dose administered in 7 subjects and for 0.90% in the other subject. Paracetamol, the main metabolite of phenacetin, was present in all samples, but its concentration was not determined.

The explanation for the very low phenacetin levels was provided by the work of Raaflaub and Dubach (1975), who determined plasma levels of phenacetin after oral as well as after intravenous administration. On basis of the i.v. experiments they could calculate the real total body clearance
of phenacetin. A clearance constant of 1–2 l/min was obtained, which is near to the hepatic bloodflow. Since the elimination proceeds almost exclusively by hepatic metabolism, a substantial first-pass effect may be expected. Raaflaub and Dubach presented evidence that the first-pass effect was diminished with increasing dose. A similar phenomenon was observed in case of salicylamide (Barr, 1969). As the authors indicated such a phenomenon is caused by capacity-limited metabolism at the first passage of the drug through the liver or even before or during absorption. In such a situation a quantitative approach yields a model and plasma curves as depicted in figure 11.1. The area under the plasma curve increases

![Figure 11.1](image_url)

**Figure 11.1**
Theoretical plasma curves demonstrating the occurrence of a saturable first-pass effect. The parameters used are based upon phenacetin data from the literature ($V_G = V_L = 5l$, $V_C = 30l$, $k_{Ca} = 15l/hr$, $k_{LC} = k_{CL} = 10l/hr$, $Q_m = 500mg/hr$, $K_M = 10mg/l$). The dose is varied and it may be noted that at the low doses the first-pass effect is very pronounced, leading to very low plasma levels. See text for further explanation.
disproportionately with increasing dose. Low doses give no appreciable plasma levels at all, so that the administration of a dose of for instance 250 mg phenacetin can only be effective as far as paracetamol is formed. From this point of view it is not sensible to use phenacetin in the form of the common combination preparations. Furthermore contrary to paracetamol (see below) the biological availability of phenacetin has been shown to be strongly dependent on the pharmaceutical formulation (Prescott et al., 1970).

b. Paracetamol

The pharmacokinetic behaviour of paracetamol has been extensively studied (Nelson and Morioka, 1963; Gwilt et al., 1963; Cummings et al., 1967; Prescott et al., 1968; Grove, 1971; Albert et al., 1974a). For the half-life average values in the range of 1.5—2.5 hrs have been reported and the volume of distribution according to one compartment kinetics may be estimated at some 50 l. Consequently the clearance constant will be in the order of 200—400 ml/min. Normally less than 5% of this total clearance consists of renal excretion.

Neither the presence of food (Jaffe et al., 1971; McGilveray and Mattok, 1972) nor the pharmaceutical formulation (Prescott, 1969; McGilveray et al., 1971; Glynn and Bastain, 1973; Richter and Smith, 1974; Albert et al., 1974b) seem to influence the biological availability negatively, although these factors may cause variations in the rate of absorption.

Methods

15 volunteers received a single oral dose of paracetamol (500 mg) in different formulations (tablet, capsule, elixir). The subjects, 13 male, 2 female were pharmacists, participating in a course on pharmacokinetics. The drug was ingested at 11.00 hr in the morning and bloodsamples (6) were taken at regular intervals for 5 hrs thereafter.

Paracetamol was determined as described in chapter 2. The concentration data were given a weight factor on basis of the accuracy of each individual value, as determined from 3—6 analyses. Taking into account the relative weights, the data were analysed by computer fit according to the program Farmfit.

Of course under the relatively uncontrolled conditions of this investigation, no real biopharmaceutical pattern can be evaluated, but if systematic variations would occur, this still might be related to the dosageform.
Results and discussion
Several examples of the plasmacurves obtained are represented in figure 11.2. The data do not allow any statement on differences in absorption rate or bioavailability. Therefore we summarize the average pharmacokinetic parameters together with their standard deviation in table 11.1. It may be noted that the absorption of paracetamol is rapid in any case and that optimal fit requires only small lagtimes, although the variation in these parameters is high. The total body clearance constant and the volume of distribution (at least their value relative to F) show remarkably little variation. This indicates that differences in biological availability will be negligible for practical purposes.

These data suggest that biopharmaceutical factors are not very critical with respect to paracetamol. Furthermore the relatively rapid absorption indicates that the analgesic effect of paracetamol may be expected to become prominent already soon after drug intake.
TABLE 11.1

Average pharmacokinetic parameters for paracetamol (n=15)

<table>
<thead>
<tr>
<th></th>
<th>V/F (1)</th>
<th>k_{el}/F (ml/min)</th>
<th>τ_{el} (min)</th>
<th>τ_{a} (min)</th>
<th>lag (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>48</td>
<td>370</td>
<td>141</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>SD</td>
<td>8</td>
<td>80</td>
<td>32</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>

Together with the fact that paracetamol is a very safe drug (in normal doses), these observations confirm the suitability of paracetamol as a general minor analgesic. As a matter of fact one might wonder if not paracetamol should be preferred over for instance acetylsalicylic acid and its derivatives in those cases where no anti-inflammatory effect is needed.

REFERENCES


The rate of elimination of drugs from the body is mainly determined by two pharmacokinetic parameters: the clearance, which is a measure for the efficiency of the eliminating organs with respect to a particular drug, and the volume of distribution, which determines how large the (apparent) volume is, from which the drug has to be cleared. Obviously both parameters will be closely related to the physicochemical properties of the drug.

Section II deals with the disposition of several antiinflammatory and antipyretic analgesics in man, in so far as their pharmacokinetic behaviour can be adequately described by linear processes. For structural reasons also one drug with a clearly non-linear elimination is included in this section, viz. 4-aminophenazone.

The pharmacokinetic data were obtained by administering normal, usual doses of the various drugs orally to young, healthy volunteers (mostly students), taking blood samples at regular intervals after ingestion, measuring plasma concentrations and renal excretion rates and analysing the measurements according to pharmacokinetic models, usually the one-compartment model.

Of course the general applicability of the figures is limited, in so far as only a selected, rather homogeneous group out of the whole population has been studied. Nevertheless our studies provide a general insight into the pharmacokinetic features of the drugs under consideration and they give information on the mechanisms of elimination involved. Furthermore the cross-over design of the investigations is highly useful for relating molecular structure with pharmacokinetic properties as well as for establishing biopharmaceutical differences between various dosage forms.

In chapter 4 the fundamentals of linear pharmacokinetics are outlined. All rate processes are assumed to be governed by linear, first-order differential equations. A general approach to the formal description of pharmacokinetic models is proposed and the relevant concepts and notions are discussed in detail. Attention is paid also to the possible accumulation of drugs on chronic dosage.

The remaining chapters of section II are devoted to the specific drugs. Ibuprofen (Brufen®) and alclofenac (Mirvan®) are the subject of chapters 5 and 8 respectively. These two drugs show a small volume of
distribution ($<10^1$), even smaller than the volume of extracellular fluid, which is probably caused by binding to plasma proteins. Also their clearance constant is rather small (30-50 ml/min), but due to the small volume of distribution the half-life of these drugs is short (1.5-3 hr). Although the total clearance of ibuprofen and alclofenac is about the same, large differences exist in the relative contributions of renal and metabolic clearance. Ibuprofen is hardly excreted unchanged in urine, whereas alclofenac may be excreted in urine for amounts up to 50% of the total dose administered. These differences are assumed to be related to differences in physicochemical properties. The lower the water solubility, the smaller the fraction of the dose excreted in urine. Further, a high lipophilicity enhances tubular reabsorption, thereby diminishing net renal excretion. Also binding to plasma proteins may be an important factor, since it lowers the plasma concentration of free drug, which is available for glomerular filtration. Especially ibuprofen is almost completely protein bound in the therapeutic concentration ranges. Contrary to alclofenac, ibuprofen exhibits clear signs of a tubular secretion process, but this is easily saturated and has only a low capacity. The renal excretion of unchanged alclofenac correlates with the urine flow: the higher the average urine flow, the larger the amount of alclofenac appearing in urine.

Both drugs are rapidly absorbed. For ibuprofen we found the time constant of absorption to be dependent on the formulation of the drug: when comparing a solution with capsules and commercial tablets the solution appears to be extremely rapidly absorbed, the absorption of ibuprofen capsules is somewhat slower and that from the commercial tablets still slower, but even for the tablets peak concentrations in plasma are usually reached within 1.5 hr. In case of alclofenac we compared tablets and suppositories, both commercial dosage forms. These appear to be equivalent for all practical purposes.

As was to be expected on basis of the single dose experiments, we confirmed that neither ibuprofen nor alclofenac tend to accumulate during chronic administration in the usual dosage regimens.

In chapter 6 the pharmacokinetics of the separate optical isomers of ibuprofen is discussed. Evidence is found to support the view, that in the human body inversion from levo- to dextro-ibuprofen occurs.

Chapter 7 describes a comparison of the human pharmacokinetics of ibuprofen and ibufenac. The volume of distribution of ibufenac seems to be somewhat larger than that of ibuprofen. It is absorbed much more slowly and usually eliminated less rapidly than ibuprofen. The elimination clearance, however, tends to be higher for ibufenac than for ibuprofen.
Indications were found for an enterohepatic circulation of ibufenac.

The topic of chapter 9 is the pharmacokinetic behaviour of flufenamic acid (Arlef®) and mefenamic acid (Ponstan®) in man. These two fenamates show rather irregular plasma curves, and it was impossible to fit the data according to simple models. Relative values for the clearance constant were obtained by measuring the area under the plasma curve. The two fenamates have a remarkably low watersolubility. Especially in case of mefenamic acid this seems to cause difficulties in the absorption process. The absorption of this drug is enhanced by food intake. We assume that this is related to the production of bile, by which the drug may be emulsified to a better absorbed form.

Chapter 10 deals with some phenazone derivatives. A typical aspect of the pharmacokinetic behaviour of phenazone and its derivatives is the large interindividual variation. This is well-known for phenazone itself but we found also for the other derivatives much more pharmacokinetic variability than for the aromatic acids, even within the rather homogeneous group of volunteers we studied.

Phenazone derivatives have a volume of distribution which is approximately equal to the volume of total body water (30-40 l) or somewhat higher for some lipophilic and less hydrophilic derivatives like 4-isopropylphenazone. In this series of compounds large differences in clearance exist, ranging from 30-50 ml/min for phenazone to 500 ml/min or more for 4-isopropylphenazone and 4-aminophenazone. Renal clearance plays only a minor role for phenazone derivatives (0-6% excreted unchanged). Introduction of an isopropyl group in phenazone leads to a much more rapid metabolism (probably oxidation of the isopropyl group). The same holds true for substitution of a dimethylamino group in phenazone, leading to 4-dimethylaminophenazone. Also here the higher clearance is caused by metabolic instability of the substituent, since metabolism predominantly occurs via N-demethylation. The first demethylated metabolite 4-methylaminophenazone tends to have a somewhat longer halflife than 4-dimethylaminophenazone. It appears that the N-methyl group is a better leaving group when it is accompanied by a second methyl group than when the amino function is monosubstituted. 4-Isopropylaminophenazone is more rapidly eliminated than 4-dimethyl- or 4-methylaminophenazone, indicating that the isopropyl group is more rapidly removed than the methyl group.

The consequences of the pharmacokinetic parameters for the rationality of some combination preparations is discussed.

The clearance of 4-aminophenazone appears to be essentially non-linear.
This substance is eliminated partly by renal excretion (in some cases up to 40%, unusually high for a phenazone derivative) and for the rest by N-acetylation. The relative contributions of renal and metabolic pathways to the overall non-linear elimination is discussed in detail with special reference to the possible occurrence of genetically determined fast and slow acetylation behaviour. Although non-linear pharmacokinetics is the subject of section III we preferred to discuss 4-aminophenazone in the context of the other phenazone derivatives.

In chapter II briefly some pharmacokinetic information concerning phenacetin and paracetamol is presented. In normal doses phenacetin usually gives only negligible plasma levels. The explanation for this phenomenon can be found in the occurrence of a substantial first-pass effect. This is not the case with paracetamol and since furthermore the absorption of phenacetin is strongly dependent on the pharmaceutical formulation, whereas that of paracetamol is almost independent of such factors it seems that the pharmacokinetic profile is strongly in favour of the use of paracetamol instead of phenacetin.
SECTION III
NON-LINEAR PHARMACOKINETICS
CHAPTER 13 LINEAR AND NON-LINEAR KINETICS OF DRUG ELIMINATION

INTRODUCTION

Pharmacokinetics approaches the behaviour of drug in man or animals as diffusion in and out various compartments and elimination from a central compartment. In general, all kinetic processes are assumed to be first-order, so that the rate of drug transfer is supposed to be directly proportional to the drug concentration in the compartments. The kinetic processes may then adequately be described by a set of linear differential equations. (linear pharmacokinetics). Elimination of drug proceeds mainly by the liver and the kidney. In these clearance organs elimination depends on the plasma flow through the organs and the extraction or filtration efficacy. In general the rate of elimination is proportional to the concentration of drug in the plasma entering the clearance organs:

\[
\frac{dQ_{el}}{dt} = \dot{V}_{Cel} \cdot C
\]

Here is \(\frac{dQ_{el}}{dt}\) the quantity of drug (e.g. mg) eliminated per unit of time (e.g. hrs), while \(C\) is the concentration of drug in the plasma and \(\dot{V}_{Cel}\) the total body clearance (e.g. 1/hr). The clearance \(\dot{V}_{Cel}\) may depend on the flow, the concentration, the condition of the organs, protein binding etc. The clearance therefore in general will not be a constant. Obviously linear kinetics is achieved only when the clearance is constant. In that case the clearance \(\dot{V}_{Cel}\) may be replaced by a clearance constant \(k_{Cel}\). If the clearance is constant the rate of elimination is directly proportional to the concentration entering the clearance organs, so that linear kinetic elimination may also be termed supply-limited elimination.

On the other hand a substance such as ethanol, in the concentration present in man following 'normal' doses, is eliminated at a constant rate (zero-order elimination) which indicates that the elimination is merely capacity-limited. Since most if not all drugs are for a major part eliminated by enzymatic conversion into metabolites it could be expected that several other drugs might show capacity-limited elimination in man (or animals). In such cases the total body clearance is not constant and proceeds via non-linear kinetics. This indeed has been confirmed, the best known example being the kinetics of salicylic acid, as analyzed in detail by Levy et al. (1972). It should be noted that also the renal excretion or part of
this may exhibit capacity-limited behaviour viz. when tubular secretion occurs.

In case of capacity-limited elimination the profile of the semi-logarithmic plasma concentration versus time curve will be dose dependent. Only under circumstances that the body may be regarded as a single compartment analytical solution of the appropriate differential equation can be obtained.

Such a solution has been given by Lundquist and Wolthers (1958), when the capacity-limited pathway is the only channel of elimination. Similar solutions have been given by Levy (1972) and Wagner (1971, 1973).

In this chapter drug kinetics will be discussed for the case that drug elimination occurs via a single metabolic pathway that is capacity-limited while simultaneously supply-limited elimination occurs. In addition a procedure will be proposed by which initial estimates of the essential kinetic parameters can be obtained from the plasma concentration curves. The theory and consequences of capacity-limited elimination via two or more metabolic pathways will be discussed in chapter 14.

**THEORY**

![Block diagrams representing linear and non-linear kinetics of elimination from a single compartment.](Image)

**Figure 13.1**

Block diagrams representing linear and non-linear kinetics of elimination from a single compartment.

I. Merely supply-limited elimination characterized by the clearance constant.
II. Merely capacity-limited elimination characterized by the enzyme constants \( K_M \) and \( \dot{Q}_m \). In fact the clearance is not constant but is maximally equal to \( \dot{Q}_m / K_M \).
III. Supply-limited and capacity-limited elimination. A fraction is eliminated by a linear clearance process at all concentrations while the rest is eliminated by a capacity-limited process. The maximum contribution to the total body clearance of this pathway is \( f \cdot k_{Cel} \).

1. **Supply-limited elimination of drug from a single compartment**
If a drug is eliminated merely be linear elimination kinetics i.e. a supply-
limited clearance process, the total body clearance is constant, so that $\hat{V}_{\text{Cel}} = k_{\text{Cel}}$. Then the following holds true for the rate of change of drug in the body in the absence of absorption processes. (See blockscheme fig. 13.1)

$$\frac{dQ}{dt} = -k_{\text{Cel}} \cdot C$$  \hspace{1cm} (13.2)

Here $dQ/dt$ is the rate of disappearance of drug (e.g. mg/h), $k_{\text{Cel}}$ the clearance constant (e.g. in l/h), $C$ the plasma concentration at any time $t$ (e.g. mg/l).

Under the supposition that the volume of distribution remains constant there is direct relationship between change in quantity $(dQ)$ and the change in the concentration $(dC)$.

The equation then may be written as follows:

$$\frac{dC}{dt} = -\frac{k_{\text{Cel}}}{V} \cdot C \quad \text{or} \quad \frac{dC}{dt} = -\frac{1}{\tau_{\text{el}}} \cdot C$$  \hspace{1cm} (13.3)

The time constant (turnover time) directly relates to the clearance constant $k_{\text{Cel}}$, provided that the clearance constant and the volume of distribution are constant.

$$\tau_{\text{el}} = \frac{V}{k_{\text{Cel}}}$$  \hspace{1cm} (13.4)

The solution of equation 13.3 is well-known:

$$\ln C = \ln A - \frac{t}{\tau_{\text{el}}}$$  \hspace{1cm} (13.5)

or

$$C = A \cdot e^{-t/\tau_{\text{el}}} = A \cdot 2^{-t/t_{1/2}}$$  \hspace{1cm} (13.6)

Here $A$ is the apparent initial concentration. This implies that $A$ depends on the boundary conditions. In case of i.v. administration $A$ equals $D/V$ and in case of enteral administration and rapid absorption as compared to elimination: $A = D \cdot F/V$. Here $D$ is the dose and $F$ the bioavailability. For reasons of simplicity $F$ will be considered to be equal to unity.

The total body clearance is the sum of the clearance constants representing the various metabolic pathways, renal excretion and possibly other elimination mechanisms (saliva, sweat, bile, lungs, faeces). In formula:

$$k_{\text{Cel}} = k_{C_r} + k_{C_m_1} + k_{C_m_2} + \ldots$$  \hspace{1cm} (13.7)

Here $k_{C_r}$ is the renal and $k_{C_m}$ the metabolic clearance constant. The latter represents various metabolic pathways, while the former represents glomerular filtration, tubular secretion and tubular reabsorption.
2. Capacity-limited elimination from a single compartment via a single metabolic pathway.

The rate of elimination then merely depends on the rate of bio-transformation via a single pathway and in the absence of absorption, may be described by use of a Michaelis-Menten equation. The clearance is not constant but concentration dependent, so:

\[ \dot{V}_{Cel} = \frac{Q_m}{K_M + C} = k_{Cel} \frac{K_M}{(K_M + C)} \]  

(13.8)

Here \( Q_m \) is the metabolic capacity of the liver enzymes involved (in mg/h) and equal to the \( V_{max} \) when only one enzyme is involved while \( K_M \) is the (apparent) Michaelis-Menten constant (mg/l), and \( k_{Cel} \) is the clearance constant at low plasma concentration (C \( \ll K_M \)).

It is obvious that for low plasma concentrations, such that C \( \ll K_M \), the disappearance rate will be again directly proportional to the plasma concentration. Then the clearance is again concentration independent (\( \dot{V}_{Cel} = k_{Cel} \)). So the total body clearance under the condition that elimination proceeds merely via a single metabolic pathway depends only on the metabolic capacity and the dissociation constant, provided that C is small as compared to \( K_M \). Then we obtain:

\[ k_{Cel} = \frac{Q_m}{K_M} \text{ while } \tau_{el} = V \cdot K_M / Q_m \]  

(13.9)

On the other hand as long as C \( \gg K_M \) it is clear that the clearance is inversely proportional to the plasma concentration.

\[ \dot{V}_{Cel} = k_{Cel} \cdot K_M / C \text{ and } \frac{dQ}{dt} = - \dot{Q}_m \]  

(13.10)

which means that elimination under those circumstances proceeds as a zero-order process. The well-known equation then becomes:

\[ C = A - t \cdot \frac{Q_m}{V} \]  

(13.11)

This obviously holds true for the disappearance of ethanol from plasma in man, following the intake of two glasses of whiskey (Lundquist and Wolthers, 1958; Haggard et al., 1941; Wagner and Patel, 1972).

Under the given conditions of equation 13.8 the differential equation governing the change of drug concentration in the body becomes:

\[ \frac{dC}{dt} = - \frac{1}{\tau_{el}} \left[ K_M / (K_M + C) \right] \cdot C \]  

(13.12)

Here the time constant \( \tau_{el} \) is related to the clearance constant at low plasma concentration according to equation 13.4.
The equation can be integrated, but the solution is implicit with respect to the plasma concentration.

\[ \ln C = \ln A + (A - C)/K_M - t/\tau_{el} \]  

(13.13)

Figure 13.2
Plasma concentration curves based on equation 13.13 showing non-linear kinetics of elimination. The volume of distribution and the metabolic capacity are kept constant \( V = 50 \text{l} \) and \( \dot{Q}_M = 50 \text{ mg/hr} \).

a. The dose is varied as indicated in the figure from 10 to 5000 mg. \( K_M \) is constant (10 mg/l). The plasma curves become flat at higher dose, but they are straight lines with a slope determined by \( \tau_{el} \) when \( C < 0.1 \, K_M \). (\( \tau_{el} = 10 \text{ hr} \)).

b. \( K_M \) is varied from 0.3 to 100 mg/l, while the dose is constant (\( D = 100 \text{ mg} \)). The whole curve becomes flatter when \( K_M \) becomes higher. For \( K_M > 10 \text{ mg/l} \) practically no capacity limitation can be seen.
In fig. 13.2 theoretical plasma concentration curves have been given, based on equation 13.13. The profile of the curve is dose dependent. However for low plasma concentrations always a straight line is obtained for which the slope is determined merely by $\tau_{el}$. Linear pharmacokinetic behaviour of course is only obtained when the plasma concentration becomes negligible with respect to $K_M$. As a rule this will require the plasma concentration to be smaller than about 0.1 $K_M$. At such low concentrations equation 13.13 reduces to the following one:

$$\ln C = \ln A^* - t/\tau_{el}$$

(13.14)

Here $\ln A^*$ refers to the apparent initial concentration obtained by extrapolating the straight line to the ordinate. See fig. 13.3. $\ln A^*$ may be derived from equation 13.13, when $C$ may be neglected with respect to $A$, so:

$$\ln A^* = \ln A + A/K_M$$

(13.15)

The apparent dissociation constant $K_M$ can be calculated from the difference between the intercept, $\ln A^*$, of the extrapolated straight line for which the slope is determined by $\tau_{el}$ and the real intercept of initial plasma concentration $\ln A$. See fig. 13.3. After transformation to decimal logarithm the following relation may be obtained:

$$K_M = A \cdot \log e / (\log A^*/A) = 0.434A/\Delta I$$

(13.16)

where $\Delta I = \log A^* - \log A$ is the difference between the extrapolated and real intercept when the data are plotted on a decimal, semi-logarithmic scale. Experimentally $\tau_{el}$ and therefore also $t\frac{1}{2}$ may be calculated from the straight part of the semilogarithmic plasma curve at sufficiently low plasma concentrations. It is therefore essential that sensitive assay procedures are available which allow us to get unambiguous data in the region where dose-independent kinetics apply. Subsequently the metabolic capacity, $Q_m$, may be calculated from $K_M$ and $k_{Cel}$ or $\tau_{el}$ and $V$:

$$\dot{Q}_m = k_{Cel} \cdot K_M \text{ or } \dot{Q}_M = V \cdot K_M/\tau_{el}$$

(13.17)

$K_M$ and $\dot{Q}_m$ may also be calculated from the time difference $\Delta t$ between the straight line of the semi-logarithmic plasma curve and a line parallel to that starting from $A$.

It can easily be derived that:

$$K_M = \frac{A\tau_{el}}{\Delta t} \text{ and } \dot{Q}_m = \frac{AV}{\Delta t}$$

(13.18)
Outline of the procedure for the initial estimation of the values of $K_M$ and $Q_m$. The parameters for the given curve are: $D = 2000 \text{ mg}$, $V = 50 \text{ l}$, $K_M = 10 \text{ mg/l}$, $Q_m = 200 \text{ mg/hr}$ and $t_{el} = 2.5 \text{ hr}$. $\Delta I$ and $\Delta t$ are explained in the text (see equations 13.16 and 13.18).

Obviously the constants $K_M$ and $Q_m$ calculated in this way bear only a relation to the real values if the requirements set before have been fulfilled. For ethanol and to some extent also for phenytoin the equation holds reasonably true so that the kinetic and enzyme parameters of these drugs have been calculated with the procedure outlined before. See table 13.1 and discussion.

It is in general unlikely that a single capacity-limited pathway is the only way of drug elimination. For most drugs supply-limited elimination will occur simultaneously with capacity-limited elimination via one or more pathways.
3. Simultaneous supply- and capacity-limited elimination from a single compartment.

If one metabolic pathway becomes capacity-limited by increasing the dose, then at the low concentration range (C ≪ K_M) a fraction, f, of the total body clearance occurs via that pathway.

The clearance or clearance function then becomes:

\[ \dot{V}_{Cel} = (1-f)k_{Cel} + \dot{Q}_m/(K_M+C) \]  \hspace{1cm} (13.19)

Here (1-f)k_{Cel} represents that part of the total body clearance that remains concentration independent while the other part f.k_{Cel} equals \( \dot{Q}_m/K_M \) only at low plasma concentration (C ≪ K_M) and decreases at higher concentrations (saturation effect).

The change in the plasma concentration now can be described by the following differential equation:

\[ \frac{dC}{dt} = -\frac{1}{\tau_{el}} \left\{ (1-f)A + f\cdot \frac{K_M}{(K_M+C)} \right\} C \]  \hspace{1cm} (13.20)

Integration with boundary condition (t=0, C=A) leads to an implicit solution for C:

\[ \ln C = \ln A + \frac{f}{1-f} \left\{ \ln \frac{1+(1-f)A/K_M}{1+(1-f)C/K_M} \right\} - \frac{t}{\tau_{el}} \]  \hspace{1cm} (13.21)

A similar differential equation and solution have been given by Wagner (1971).

Theoretical plasma concentration curves for a drug with K_M = 3 mg/l are shown in figure 13.4. Clearly at low plasma concentration (C ≪ K_M) always parallel straight lines are obtained of which the slope is solely determined by \( \tau_{el} \). However, at very high plasma concentrations also straight lines are obtained which are flatter.

The concentration-dependent component \( \dot{Q}_m/(K_M+C) \) in the apparent clearance progressively becomes less important with respect to the concentration-independent component (1-f)k_{Cel} by increasing the plasma concentration. Obviously this will occur earlier as f is smaller. It is worthwhile to consider first the situation that the plasma concentration is large with respect to K_M but that \( \dot{Q}_m/C \) is not yet negligible to (1-f)k_{Cel}. In that case equation 13.19 reduces to:

\[ \dot{V}_{Cel} = (1-f)k_{Cel} + \frac{\dot{Q}_m}{C} \]  \hspace{1cm} (13.22)

and equation 13.20 becomes
Plasma concentration curves in case of combined linear and non-linear kinetics of elimination. The volume of distribution and the metabolic capacity of the saturable system are kept constant: $V = 50 \text{l}$ and $Q_m = 50 \text{mg/hr}$.

a. Dose variation from 100 to 5000 mg for a theoretical drug that for 2/3 is eliminated by a potentially capacity-limited pathway ($f = 0.67$), $K_M = 3 \text{mg/l}$ and $\tau_{el} = 2 \text{hr}$. At concentrations below 0.1 $K_M$ the curves are parallel.

b. Variations of the fraction of the total body clearance that proceeds via a capacity-limited pathway, from $f = 0.2$ to $f = 1$. The clearance constant $k_{Cel}$ is kept at 25 l/hr, which means that $\tau_{el} = 2 \text{hr}$. So $K_M$ is varied along with $f$ according to:

$$K_M = \frac{\dot{Q}_m}{f k_{Cel}} = \frac{2}{f} \text{mg/l}$$

It is clear that capacity-limitation will not be detected when $f < 0.2$.

$$\frac{dC}{dt} = -\frac{1}{\tau_{el}} \left( (1-f) + \frac{fK_M}{C} \right) C = -\frac{(1-f)C}{\tau_{el}} - \frac{fK_M}{\tau_{el}}$$

(13.23)

Integration with boundary condition ($t=0, C=A$) leads to:

$$C = Ae^{-t/\tau^*_el} - \frac{f}{1-f} K_M (1-e^{-t/\tau^*_el})$$

(13.24)

Where $\tau^*_el = \frac{\tau_{el}}{1-f}$ is the time constant corresponding with the dose-independent part of the clearance $(1-f)k_{Cel}$. This equation describes the plasma concentration curve for concentrations about 10 times $K_M$. The influence
of the second term in the equation becomes progressively less the more \( C \) is larger with respect to \( K_Mf/(1-f) \). In practice the equation at sufficiently high concentration values will appear as a nearly linear line on semilog plot, the slope of which will be close to \( 1/\tau_{el}^* \). This may easier be seen from equation 13.22, where ultimately \( Q_m/C \) may be neglected with respect to \( (1-f)k_{Cel} \), so that a concentration independent clearance results. Therefore at very high plasma concentrations elimination is also merely supply-limited. In this extreme case the clearance process can be simply described as:

\[
\dot{V}_{Cel} = (1-f)k_{Cel}
\]

so

\[
\frac{dC}{dt} = -\frac{C}{\tau_{el}^*} = -(1-f)\frac{C}{\tau_{el}}
\]

which upon integration leads to:

\[
\ln C = \ln A - \frac{t}{\tau_{el}^*}
\]

So in general the contribution of the capacity-limited pathways can be calculated from the ratio of the slopes of the two straight line segments in a semilogarithmic plasma concentration-time plot. It should, however, be realized that the correct slope for \( \tau_{el}^* \) is only found at very high plasma concentrations. Consequently \( f \) will be underestimated from most experimental data. See discussion. When \( f = 0.5 \), so that 50% of the total body clearance (at \( C \ll K_M \)) occurs via a potentially capacity-limited pathway, the limiting slope at high plasmaconcentrations is a factor 2 less than the slope in the low concentration range where linear kinetics prevail, but the correctness of the estimation will be strongly dependent on the number of plasma data available and the height of the dose given. If however, the potentially capacity-limited pathway contributes to less than 20% to the overall clearance the difference in slope is hardly accessible. See fig. 13.4.

This implies that capacity-limited elimination pathways may easily be overlooked. However, a capacity-limited pathway which contributes little to the overall body clearance may be of importance with regard to interference with the elimination of other drugs which are metabolized via the same enzymatic processes.

The straight line at low plasma concentrations (\( C \ll K_M \)) is given by the following equation which is derived from equation 13.21 by neglecting \( C \) with regard to \( K_M \):
\[
\ln C = \ln A + \frac{f}{1-f} \ln \left[ 1 + (1-f) \frac{A}{K_M} \right] - \frac{t}{\tau_{el}} \quad (13.28)
\]

The intercept of this straight line, \( \ln A^* \), is larger than the real intercept and can be represented by the following equation, analogous to equation 13.15

\[
\ln A^* = \ln A + \frac{f}{1-f} \ln \left[ 1 + (1-f) \frac{A}{K_M} \right] \quad (13.29)
\]

Since \( f \) can be calculated from the ratio in the slopes of the straight line segments at very high (\( C > K_M \)) and at very low (\( C < K_M \)) plasma concentrations, \( K_M \) may be calculated from the difference between the real and the extrapolated intercepts in a semilog plot according to the following relation, obtained from equation 13.29, by rearrangement and transformation to decimal logarithms:

\[
\Delta I = \frac{f}{(1-f)} \log \left[ 1 + (1-f)A/K_M \right] \quad (13.30)
\]

The apparent Michaelis-Menten constant, \( K_M \), can also be calculated from the time shift \( \Delta t \) between the straight line according equation 13.28 and a parallel straight line starting at \( A \). It can be derived that

\[
\Delta t = \frac{f \cdot \tau_{el}}{(1-f) \log e} \log \left[ 1 + (1-f)A/K_M \right] \quad (13.31)
\]

Once \( K_M \) has been determined, also the metabolic capacity of the enzyme system concerned, becomes quantitatively accessible on the basis of:

\[
\dot{Q}_m = f \cdot k_{Cel} \cdot K_M = f \frac{V}{\tau_{el}} \cdot K_M \quad (13.32)
\]

Some examples of combined capacity- and supply-limited elimination are shown in fig. 13.5. Salicylic acid has been administered orally as a solution but absorption is very fast with respect to elimination so that equation 13.21 in good approximation holds true. Parameters calculated according to the procedure outlined before are summarized in table 13.1.

Since experimentally \( f \) is underestimated the initial values of \( K_M \) and \( \dot{Q}_m \) obtained will not be optimal. Therefore the initial values obtained for \( K_M \) and \( \dot{Q}_m \) have been used in a non-linear curve-fitting computer program in order to obtain more reliable values of the in vivo enzyme parameters. From table 13.1 it may be seen that the initial values that follow from the procedure outlined above, give a reasonable estimation of the values obtained by curve-fitting.
**DISCUSSION**

In general the main importance of graphical methods for estimation of pharmacokinetic parameters lies in the possibility of obtaining initial estimates which can be used for subsequent digital computer fitting procedures. Of course the precision of these initial approximations is dependent on the number of experimental data available and the accuracy and the sensitivity of the method used for the assay of the plasma concentration. For instance in the well-known method of residuals for compartmental analysis it is essential to have enough data for correctly estimating the slope of the extrapolated straight lines. Furthermore deeper compartments can easily be overlooked when plasma concentration has not been followed long enough. At least the same degree of accuracy, sensitivity and
specificity of the assay method is required for optimal application of the procedure outlined above. For the most simple model of capacity-limited elimination (equations 13.12 - 18) where the saturable metabolic pathway is the only mechanism of elimination the graphically estimated values for \( K_M \) and \( Q_m \) may be a good approximation of the real value, provided that the slope of the straight line at low plasmaconcentrations (so \( r_{el} \)) and by that the extrapolated intercept \( A^* \) is accurately determined. As a matter of fact this straight line can only be estimated from concentration data in the region where the plasmalevels are negligible with respect to \( K_M \). When enough data are available below 0.1 \( K_M \) it will be possible to obtain \( K_M \) and \( Q_m \) values with an error of only a few per cent. Of course the magnitude of the deviation of the estimated parameters from the real ones will become larger when the plasmaconcentration has not been followed long enough. Nevertheless the method will always indicate the order of magnitude of the parameters and provide an initial estimate for a subsequent computer fitting. As far as the second model, with simultaneous supply- and capacity-limited elimination is concerned another complicating factor may arise. The estimated value of \( K_M \) is dependent, not only on \( \Delta \), the accuracy of which is determined by the factors discussed above, but also on \( f \). This fraction \( f \) is estimated from the ratio of the slopes of the two straight lines which arise at very high and at very low plasma concentration. Now \( \tau_{el}^* \) can only be determined accurately at such high plasma concentrations that contribution of the saturated mechanism to the overall elimination is negligible with respect to the contribution of the mechanism(s) that remains(s) linear over the whole experimental concentration range. It is doubtful whether this situation will ever be reached at all. For instance the maximum dose that can safely be administered may be a serious restriction in this respect. Therefore the estimated values for the parameters according to the model with combined capacity- and supply-limited mechanism as a rule will deviate more from the real values than in the case of the simplest model. Once again it should be stressed that computer fitting starting with the initial graphical estimates of the parameters will greatly improve the accuracy of the approximations.

In the kinetic parameters calculated from the experimental data from our laboratory and the literature certain errors may have been introduced. In the case of ethanol from data by Wagner and Patel (1972) it is assumed that absorption is both complete and rapid with respect to elimination. These suppositions seem to be reasonable. Ethanol is known to be oxidized in man for 90 to 98% so that \( f \) is practically unity. However, two distinct mechanisms are available: the liver alcoholdehy-
### TABLE 13.1

Estimated and computer fitted parameters for some drugs that show capacity-limited elimination

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ethanol</th>
<th>Salicylic acid</th>
<th>Phenytoin</th>
<th>4-hydroxybutyric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D (mg)</strong></td>
<td>48000(^a)</td>
<td>64000(^b)</td>
<td>770(^c)</td>
<td>1155(^c)</td>
</tr>
<tr>
<td><strong>A(^{est}) (mg/1)</strong></td>
<td>6,6.10(^e)</td>
<td>7,2.10(^g)</td>
<td>600</td>
<td>2500</td>
</tr>
<tr>
<td><strong>A(^{est}) (mg/1)</strong></td>
<td>1330</td>
<td>1220</td>
<td>125</td>
<td>200</td>
</tr>
<tr>
<td><strong>A(^{fit}) (mg/1)</strong></td>
<td>1310</td>
<td>1320</td>
<td>129</td>
<td>191</td>
</tr>
<tr>
<td><strong>V(^{fit}) (1)</strong></td>
<td>36,7</td>
<td>48,5</td>
<td>5,98</td>
<td>6,0</td>
</tr>
<tr>
<td><strong>(1)</strong></td>
<td>(1,7)</td>
<td>(1,4)</td>
<td>(0,16)</td>
<td>(0,8)</td>
</tr>
<tr>
<td><strong>(r) (hr)</strong></td>
<td>-</td>
<td>-</td>
<td>7,2</td>
<td>9,7</td>
</tr>
<tr>
<td><strong>(k) (hr(^{-1}))</strong></td>
<td>-</td>
<td>-</td>
<td>0,14</td>
<td>0,10</td>
</tr>
<tr>
<td><strong>(r) (hr)</strong></td>
<td>0,43</td>
<td>0,68</td>
<td>3,3</td>
<td>3,3</td>
</tr>
<tr>
<td><strong>(k) (hr(^{-1}))</strong></td>
<td>2,33</td>
<td>1,47</td>
<td>0,30</td>
<td>0,30</td>
</tr>
<tr>
<td><strong>(V) (hr)</strong></td>
<td>0,42</td>
<td>0,60</td>
<td>2,8</td>
<td>2,5</td>
</tr>
<tr>
<td><strong>(0,06)</strong></td>
<td>(0,08)</td>
<td>(0,3)</td>
<td>(0,6)</td>
<td>(0,8)</td>
</tr>
<tr>
<td><strong>(0,05)</strong></td>
<td>(0,04)</td>
<td>(0,06)</td>
<td>(0,08)</td>
<td>(0,10)</td>
</tr>
<tr>
<td><strong>K(_{cal}) (hr(^{-1}))</strong></td>
<td>2,38</td>
<td>1,67</td>
<td>0,36</td>
<td>0,4</td>
</tr>
<tr>
<td><strong>K(_{est})</strong></td>
<td>-</td>
<td>-</td>
<td>0,54</td>
<td>0,66</td>
</tr>
<tr>
<td><strong>K(_{fit}) (1)</strong></td>
<td>-</td>
<td>-</td>
<td>0,79</td>
<td>0,91</td>
</tr>
<tr>
<td><strong>(0,09)</strong></td>
<td>(0,16)</td>
<td>(0,16)</td>
<td>(0,16)</td>
<td>(0,16)</td>
</tr>
<tr>
<td><strong>K(_{M, est}) (mg/1)</strong></td>
<td>210</td>
<td>190</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td><strong>K(_{M, fit}) (mg/1)</strong></td>
<td>200</td>
<td>140</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td><strong>(45)</strong></td>
<td>(30)</td>
<td>(17)</td>
<td>(30)</td>
<td>(4)</td>
</tr>
<tr>
<td><strong>(1)</strong></td>
<td>27,1</td>
<td>43,4</td>
<td>6,0</td>
<td>6,0</td>
</tr>
<tr>
<td><strong>(1/hr)</strong></td>
<td>64,5</td>
<td>72,3</td>
<td>2,1</td>
<td>2,4</td>
</tr>
<tr>
<td><strong>Q(_{m, cal}) (mg/hr)</strong></td>
<td>12900</td>
<td>10100</td>
<td>44</td>
<td>59</td>
</tr>
</tbody>
</table>
Explanation of the symbols used in Table 13.1.
The symbols $A^*$, $A$, $V$, $\tau$, $k$, $f$, $K_M$, $k_{cel}$ and $\dot{Q}_m$ are explained in the text. The suffixes est, fit and cal mean 'estimated', 'fitted' and 'calculated' respectively. 'Estimated' refers to the graphical parameter determination, 'fitted' are the values directly obtained from the computer fitting program and 'calculated' values are derived from estimated and fitted parameters according to the equations described in the text.

$V_{est}$ is the best possible estimation of the actual volume of distribution and is used for calculations of $k_{cel,cal}$ and $\dot{Q}_m,cal$, while $V_{fit}$ is just an operational magnitude, leaving out of consideration distribution or absorption processes, and used for descriptions according to eq. 13.13 or 13.21.

Legend to Table 13.1

a. Data from Wagner and Patel (1972). For estimation of the parameters as well as for the curve fitting the zero-time has been taken after absorption can be expected to be completed. Therefore the intercept $A$ and the volume $V_{fit}$ do not correspond to the actual volume of distribution $V_{est}$, which has been estimated for the whole plasma concentration curve.
b. Data from Haggard et al. (1941). Same remarks as under a.
c. Two oral doses (potto) were given to the same subject. Absorption is very rapid (see fig. 13.5). Therefore a close agreement exists between $V_{est}$ and $V_{fit}$ and the curves can be handled as if i.v. administration was applied.
d. Data from Glazko et al. (1969). Based on mean plasma concentration values in 6 persons who received 250 mg of phenytoin by i.v. infusion. Concentration points in the distribution phase have been left out when estimating the parameters or fitting the curve.
e. Data from v.d Pol et al. (1975) in three patients following i.v. infusion. The distribution phase has been left out for estimation of the parameters and curve fitting.
f. The magnitude of $A_{fit}$ has been calculated from $V_{fit}$ and the dose $D (A = \frac{D}{V})$ since by the computer program used the experimental points are fitted to an equation in which $A$ is written explicitly as $\frac{D}{V}$ so that $V$ is fitted directly.
g. The number in parentheses represents the error in the fitted parameter value. See text for further explanation. Errors are only given for parameters that were directly fitted and not for those that were calculated from fitted values, since such errors would hardly be meaningful.
drogenase and the liver microsomal oxidizing system. The relative con-
tribution of these systems to the overall metabolism of ethanol are de-
dependent on the plasma concentration that exists.

Some problems arise with regard to the calculation of the kinetic para-
eters in this case. As will be discussed in chapter 14 an apparent \( K_M \) is
expected to come out, which is a function of the two \( K_M \) values for both
systems and their relative importance or weight. For the moment it is
sufficient to note that the \( K_M \) value given in table 13.1 is in the expected
order of magnitude. The two metabolic systems have different \( K_M \) values
as follows from in vitro studies. For alcohol-dehydrogenase \( K_M \) is about
80 mg/l and for the microsomal oxidizing system \( K_M \) is about 400 mg/l
(Lieber, 1973). Important parameters obviously are the metabolic capacities
of the two systems concerned, for which unfortunately no information is
available. In our analysis with regard to ethanol we now used the simplest
model. It should be noted that the calculated metabolic capacity \( Q_m \) seems
to be a very reasonable overall estimate. As far as \( K_M \) is concerned however
one must bear in mind that the calculated values actually may be the result
of some combination of two constants. Further analysis can only be done
when more accurate data become available.

Phenytoin seems to be hydroxylated for about 80% (Glazko et al.,
1969). Assuming this to be due to a saturable metabolic system, one
would expect the fraction \( f \) to be about 0.8. However a good computer
fitting of the data requires \( f=1.0 \), as shown in table 13.1 so we are forced
to conclude that the data are not totally sufficient for discriminating
between the several possibilities.

In case of salicylic acid, absorption form the oral route is fast with
respect to elimination, but at least two capacity-limited pathways are in-
volved. This implies that the procedure according to equation 13.21 may
not be applied unless additional requirements have been fulfilled. When
two different capacity-limited pathways occur simultaneously with a con-
centration independent fraction, the mathematical relationship between
plasma concentration and time becomes more complicated. The calcula-
tion of the kinetic parameters is not straight forward anymore.

However, in the case of salicylic acid elimination in man the \( K_M \) values
of the two metabolic systems that are easily saturated, are in the same
order of magnitude as may be seen from renal excretion data by Levy et
al. (1972).

It may be calculated that if two saturable pathways are involved with the
same \( K_M \) value the differential equation may be written as a single path-
way while the overall capacity is the sum of the two capacities. Our data

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do not allow a more detailed analysis. The overall values obtained for salicylic acid from plasma data agree reasonably with the data obtained from renal excretion of metabolites by Levy et al. (1972)

The errors in the kinetic and enzyme parameters as given in table 13.1 reflect the goodness of fit of the data to the model. When many data are used this error can be regarded as the standard error. In the cases that are described here however the number of experimental data is certainly not enough with respect to the number of parameters to be fitted, so that the exact meaning of the value of the error is somewhat obscure. Of course the error is strongly dependent upon the number of data available and this may contribute to the fact that some parameters show a rather large error although on inspection the fitted curves excellently adapt the experimental points. As a matter of fact the magnitude of the error in a parameter reflects the degree of sensitivity of the fitted curve to changes in that parameter. The error in $K_M$ and $\tau_{el}$ might still be enlarged by the strong positive correlation which appears to exist between these two parameters.

In general non-linear pharmacokinetics deals with all processes that deviate from first-order kinetics. There are, however, only a few processes for which a better description than first-order approximation is possible. These are drug metabolism (as discussed in the foregoing paragraphs), some types of renal excretion and drug-protein binding.

**Renal excretion**

In the kidney two basic processes mostly play an important role in drug excretion: glomerular filtration and passive reabsorption. These two processes may be summed up to yield one linear renal clearance constant. Two other saturable, so non-linear, mechanisms may be relevant for the elimination of a number of special drugs: tubular secretion (e.g. for many aromatic acids) and sometimes active tubular reabsorption (e.g. glucose, uric acid). The tubular secretion again can be related to the plasma concentration by an equation totally analogous to equation 13.8. The quantity of drug eliminated per unit of time by a tubular secretion process can be written as:

$$\frac{dQ_T}{dt} = \frac{T_M}{K_T + C} \cdot C \quad (13.33)$$

where
Some characteristics of tubular secretion have been discussed in chapters 5, 8 and 10.

Drug-protein binding
Of course drug-protein binding will not influence the intrinsic clearance of a drug (calculated on basis of free drug concentration) but only the apparent clearance (on basis of total plasma concentration). When a certain drug, which is strongly bound to plasma proteins, can achieve such high concentrations that it exceeds the protein-binding capacity there will be a disproportional increase in free drug concentration, available for diffusion. The excess is causing an increase in the apparent volume of distribution, which automatically will lead to a directly proportional increase in apparent total body clearance.

Figure 13.6 shows the relationship between clearance and drug concentration for some theoretical drugs with different degree of protein binding. It is important to notice that in any case the clearance is constant over substantial concentration ranges, so that the effect of the protein binding may be detected clearly only in a few instances. Furthermore, it is interesting to see that the effect of protein binding is just opposite to that of saturable elimination (see fig. 13.7).

As a matter of fact one might wonder if not in many cases capacity-limited elimination cannot be detected since it is balanced by the consequence of drug-protein binding. A very nice experimental example of the influence of protein binding on pharmacokinetic behaviour can be found in the work of Burns et al. (1953) who found that increasing the daily dose of phenylbutazone in man (200, 400, 800 to 1600 mg) led to a much less than proportional increase in plasma plateau concentration. This is exactly as would be expected for a strongly protein-bound drug in high concentrations: increase in dosage effectively counterbalanced by a concomitant increase in apparent total body clearance. The influence of drug-protein binding on drug distribution and elimination has been discussed for instance by Krüger-Thiemer et al. (1966), Dayton et al. (1973) and Schoenemann et al. (1973).

Finally it should be noticed that description of plasma concentration curves in terms of Michaelis-Menten formulas implies the presupposition of
an equilibrium between the drug concentration in plasma and in the compartment where metabolic conversion or tubular secretion takes place. We cannot be sure that this assumption is justified for all drugs.

\[ V_{\text{cel}} : \text{Apparent total body clearance (l/h)} \]

\[ C : \text{plasma concentration (mg/l; log scale)} \]

Figure 13.6
Apparent total body clearance as a function of plasma concentration with different degrees of drug-protein binding for a hypothetical drug (MW 320). Equation according to Krüger-Thiemer et al. (1966).

\[ V_{\text{cel}} = k_{\text{cel}} \left[ (1-f) + fC_f/C \right] \]
\[ C = C_f \left[ \omega + Q_{\text{bm}} / (K_{\text{AP}} + C_f) \right] \]

Figure 13.7
Apparent total body clearance as a function of plasma concentration and \( K_M \) for a hypothetical drug (MW 320). Elimination is assumed to occur via parallel supply- and capacity-limited pathways.
APPENDIX I

DERIVATION OF EQUATIONS 13.18 AND 13.31

With respect to figure 13.3 one can conceive of two points with the same concentration, one on the straight line starting from log $A^*$ and one on the parallel line from log $A$, which are a time distance $\Delta t$ apart. One can write for these points:

$$\ln C = \ln A^* - \frac{t}{\tau_{el}} = \ln A + \frac{A}{K_M} - \frac{t}{\tau_{el}} \quad (13.14/15)$$

and

$$\ln C = \ln A - \frac{t - \Delta t}{\tau_{el}} = \ln A - \frac{t}{\tau_{el}} + \frac{\Delta t}{\tau_{el}} \quad (13.5a)$$

From comparison we see that

$$\frac{A}{K_M} = \frac{\Delta t}{\tau_{el}} \quad \text{so} \quad K_M = \frac{A \tau_{el}}{\Delta t} \quad (13.18)$$

Essentially the same procedure can be applied for deriving equation 13.31. Then the two points are given by:

$$\ln C = \ln A^* - \frac{t}{\tau_{el}} = \ln A + \frac{f}{1-f} \ln \left( 1 - \frac{(1-f)A}{K_M} \right) - \frac{t}{\tau_{el}} \quad (13.28/29)$$

and

$$\ln C = \ln A - \frac{t}{\tau_{el}} + \frac{\Delta t}{\tau_{el}} \quad (13.5a)$$

From comparison it follows that

$$\frac{\Delta t}{\tau_{el}} = \frac{f}{1-f} \ln \left( 1 + \frac{(1-f)A}{K_M} \right) \quad \text{or}$$

$$\Delta t = \frac{f \tau_{el}}{(1-f) \log e} \log \left( 1 + \frac{(1-f)A}{K_M} \right) \quad (13.31)$$
APPENDIX II

INTEGRATION OF EQUATION 13.20

\[
\frac{dC}{dt} = -\frac{1}{\tau_\text{el}} (1-f + \frac{fK_M}{K_M + C}) C
\]  

(13.20)

Rearrangement leads to

\[
\frac{K_M + C}{[K_M + (1-f) C]} \frac{dC}{dt} = \left[ \frac{1}{C} + \frac{f}{K_M + (1-f) C} \right] dC = \frac{dt}{\tau_\text{el}}
\]  

(13.34)

Now integration is straightforward and, with the boundary conditions \((t=0, C=A)\) the solution is equation 13.21.

APPENDIX III

Equation 13.13 is just a special form of equation 13.21, viz. for the case where \(f=1\). Equation 13.21 reduces to equation 13.13 as \(f\) approaches unity. Starting with equation 13.21 we have to calculate

\[
\lim_{f \to 1} (\ln C) = \ln A - \frac{t}{\tau_\text{el}} - \lim_{f \to 1} \left[ \frac{f}{1-f} \ln \frac{1 + (1-f) C/K_M}{1 + (1-f) A/K_M} \right]
\]  

(13.35)

Simple rearrangement gives

\[
\lim_{f \to 1} \left[ \frac{f}{1-f} \ln \frac{1 + (1-f) C/K_M}{1 + (1-f) A/K_M} \right] = \lim_{f \to 1} \left[ \frac{f}{1-f} \ln \left( 1 + \frac{(1-f) (C-A)}{K_M + (1-f) A} \right) \right]
\]

Since the following equation obviously holds true

\[
-1 \leq \frac{(1-f) (C-A)}{K_M + (1-f) A} \leq 0
\]  

(13.37)

the logarithm in equation 13.36 can be expanded into a Taylor series, so that equation 13.36 can be written as:

\[
\lim_{f \to 1} \left[ \frac{f}{1-f} \left( \frac{(1-f) (C-A)}{K_M + (1-f) A} - \frac{(1-f)^2 (C-A)^2}{2 [K_M + (1-f) A]^2} + \ldots \right) \right] = \lim_{f \to 1} \left[ \frac{f (C-A)}{K_M + (1-f) A} - \ldots \right]
\]

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\[
- \frac{f (1-f) (C-A)^2}{2 [K_M + (1-f)A]^2} + \ldots = \frac{C-A}{K_M} \tag{13.38}
\]

By substituting this result into equation 13.35 we obtain equation 13.13.

REFERENCES


CHAPTER 14 TWO CAPACITY-LIMITED PATHWAYS OCCURRING SIMULTANEOUSLY

INTRODUCTION

In chapter 13 equations have been given for the plasma concentration as a function of time for drugs that show capacity-limited elimination via a single pathway as well as supply-limited (linear) elimination. The equations appeared to be quite satisfactory for describing plasma curves for several drugs. However, as indicated already, evidence is available that at least for salicylic acid and ethanol two non-linear pathways are involved. In this chapter the appropriate equations will be derived for elimination via two capacity-limited pathways with or without simultaneous supply-limited elimination and the possibility of distinguishing the routes will be discussed. Analogously to the symbols used in chapter 13 we will refer to the capacities of the two non-linear mechanisms as \( \dot{Q}_{m1} \) and \( \dot{Q}_{m2} \) to their Michaelis-Menten constants as \( K_{M1} \) and \( K_{M2} \) and to their relative contribution to the maximal clearance as \( f_1 \) and \( f_2 \). Again \( k_{Cel} \) represents the maximal clearance constant, so obviously \( \dot{Q}_{m1} = f_1 K_{M1} k_{Cel} \) and \( \dot{Q}_{m2} = f_2 K_{M2} k_{Cel} \). The time constant for elimination when no saturation occurs at all is \( r_{el} (= \frac{V}{k_{Cel}}) \).

TWO CAPACITY-LIMITED PATHWAYS ALONE

If a drug is eliminated merely via two metabolic (or secretory) routes that can be saturated at the prevailing levels of the plasma concentration, the following differential equation describes the elimination process (in a one compartment model with i.v. administration):

\[
\frac{dC}{dt} = - \left[ \frac{f_1 K_{M1}}{K_{M1} + C} + \frac{f_2 K_{M2}}{K_{M2} + C} \right] \frac{C}{r_{el}}
\]  

(14.1)

Since here the two non-linear mechanisms are the only channels of elimination, it is obvious that \( f_1 + f_2 = 1 \).

Equation 14.1 can be integrated, leading to a solution which is implicit in \( C \). With the boundary condition \( [t=0; C=A (= \frac{D}{V})] \), one obtains (see also appendix):
\[ \ln C = \ln A + \frac{A - C}{f_1 K_{M1} + f_2 K_{M2}} - \frac{t}{\tau_{el}} + \]

\[ + \frac{f_1 f_2 (K_{M1} - K_{M2})^2}{(f_1 K_{M1} + f_2 K_{M2})^2} \ln \frac{A(f_1 K_{M1} + f_2 K_{M2}) + K_{M1} K_{M2}}{C(f_1 K_{M1} + f_2 K_{M2}) + K_{M1} K_{M2}} \]

(14.2)

When \( K_{M1} \) would be equal to \( K_{M2} \), equation 14.2 clearly reduces to equation 13.13. Of course also on basis of equation 14.2 ultimately a straight line is obtained in a semilogarithmic plasma curve (viz. when C is very small with respect to the smallest \( K_{Mj} \)). The slope of this straight line is determined solely by \( \tau_{el} \). From equation 14.2 one can easily derive expressions for timeshift \( \Delta \) by procedures similar to those outlined in chapter 13. Unfortunately these expressions are not practically useful, since they do not allow separate estimation of \( f_1, f_2, K_{M1} \), and \( K_{M2} \). It should be noticed that it is virtually impossible to obtain such estimates on basis of the plasma curve alone.

**TWO CAPACITY-LIMITED COMBINED WITH ONE OR MORE SUPPLY-LIMITED PATHWAYS**

In general it is unlikely that a drug would be eliminated exclusively via mechanisms that are saturated at normal plasma concentrations. Usually other mechanisms of elimination will contribute to the clearance process as well. These 'secondary' mechanisms may be less specific and have low affinity for the specific drug, but especially when high drug levels are sustained for longer periods, they can become quantitatively important. In the case that two capacity-limited mechanisms are accompanied by one or more supply-limited routes, the plasma concentration as a function of time in a one compartment model with i.v. administration is governed by the following differential equation:

\[ \frac{dC}{dt} = - \left[ 1 - f_1 - f_2 + \frac{f_1 K_{M1}}{K_{M1} + C} + \frac{f_2 K_{M2}}{K_{M2} + C} \right] \frac{C}{\tau_{el}} \]  

(14.3)
Integration yields the following equation implicit in \( C \) (see also appendix):

\[
\ln C = \ln A + \frac{K_{M1} + K_{M2} + p + (1-f_1-f_2) q}{(1-f_1-f_2)(p-q)} \ln \frac{A-p}{C-p} \\
+ \frac{K_{M1} + K_{M2} + q + (1-f_1-f_2) p}{(1-f_1-f_2)(q-p)} \ln \frac{A-q}{C-q} - \frac{t}{\tau_{el}} \tag{14.4}
\]

where \( p \) and \( q \) are the roots of the following quadratic equation:

\[
x^2 + \frac{(1-f_2)K_{M1} + (1-f_1)K_{M2}}{1-f_1-f_2} x + \frac{K_{M1}K_{M2}}{1-f_1-f_2} = 0 \tag{14.5}
\]

It can easily be shown that the discriminant of equation 14.5 is always \( > 0 \), so that the equation has always two real, different roots. With regard to equation 14.4 the same remarks can be made as for equation 14.2. The ultimate straight line which is obtained in a semilogarithmic plot is determined exclusively by \( \tau_{el} \) and it is virtually impossible to obtain reliable estimates for the various enzymatic parameters on the basis of plasma concentrations alone.

**DISCUSSION**

It has been shown in the foregoing paragraph that relatively simple integrated equations are obtained for the plasma concentration in case of elimination via two capacity-limited pathways with or without simultaneous supply-limited pathways. As a matter of fact even certain more complicated models can be analysed by the same mathematical methods as described in the appendix. In principle these integrated equations can be used for computer fit of experimental data to the models under discussion. It appears, however, that complicating the model by addition of extra parameters as in equations 14.2 and 14.3 does not lead to special characteristics in the theoretical plasma curves obtained by which these could be distinguished from the simpler equations in the foregoing chapter. When for instance theoretical data are generated on basis of equation 14.2 one finds that this simulation can easily be adapted to the much simpler equation 13.13. The same holds true for equation 14.4 with respect to
equation 13.21. As an illustrative example we simulated the model of equation 14.2 on basis of $K_M$ values as estimated for ethanol by independent procedures (90 ml/l and 400 mg/l, Lieber, 1973). Since it is obvious that the relative contributions of the two mechanisms (alcoholdehydrogenase and the microsomal oxidative system) are dependent upon the plasma concentration range studied we inserted variable values for $f_1$ (and consequently $f_2$, which is $1-f_1$). At first sight already it is clear that no characteristic features at all are present in the plasma curves and that they are highly similar to those obtained from equation 13.13 (see figure 14.1).

![Figure 14.1](image)

**Figure 14.1**

*Some examples of plasma curves simulated on basis of the simultaneous occurrence of two capacity-limited pathways of elimination, according to equation 14.2 The parameters are $K_{M1} = 90$, $K_{M2} = 400$ mg/l and $t_{el} = 30$ min (based on estimates for ethanol). The fractions $f_1$ and $f_2$, representing the relative contribution of the two pathways, are varied. From the left to the right $f_1 = 0$, $f_1 = 0.2$, $f_1 = 0.5$, $f_1 = 0.8$ and $f_1 = 1$ (the sum of $f_1 + f_2 = 1$) See text for further explanation.*

When from the simulated curve a lot of concentration data (about 40), at regular intervals, so describing the whole curve, are taken and when these data are subjected to computer fit according to equation 13.13 an excellent fit is obtained Table 14.1 shows some examples of this. It may be
TABLE 14.1

Some typical results obtained when data, generated according to equation 14.2 (with \( D = 50 \text{ g, } V = 50 \text{ l} \)) are fitted on basis of equation 13.13 (which yields a pooled \( K_M \) value).

<table>
<thead>
<tr>
<th>Parameters used for simulation(^1)</th>
<th>( f_1K_1 + f_2K_2 )</th>
<th>Fit according to eq. 13.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f )</td>
<td>( K_{M1} )</td>
<td>( K_{M2} )</td>
</tr>
<tr>
<td>0.2</td>
<td>90</td>
<td>400</td>
</tr>
<tr>
<td>0.6</td>
<td>90</td>
<td>400</td>
</tr>
<tr>
<td>0.8</td>
<td>90</td>
<td>400</td>
</tr>
</tbody>
</table>

1. \( K_M \) in mg/l; \( \tau_{el} \) in min.

noted that the error in the fitted parameters is so small that experimental data will never allow a more thorough analysis. When the same data are fitted according to equation 14.2 also a good fit can be obtained, at least when the start guess for the fitting procedure is good. This implies that in practice at least two major difficulties would be encountered. Firstly, experimental data can never be as exact as the simulated data are and secondly, as stated above, there is no simple way for obtaining estimates that are useful as starting values for the computer fit. The apparent \( K_M \) which is obtained by the pooling of two capacity-limited pathways has a value intermediate between the two separate \( K_M \)'s and is closer to one of these as the contribution of the mechanism to which \( K_M \) belongs, is increasing. From the table it becomes clear that the apparent \( K_M \) is reasonably approximated by the weighted average \( f_1K_{M1} + f_2K_{M2} \). In our example we used \( K_M \) values of 90 and 400 mg/l, the difference being a factor 4–5. This difference is much larger than the difference between the \( K_M \) values for salicylic acid with respect to its two elimination pathways that are saturable at therapeutic plasma levels (formation of salicyluric acid and of phenolic glucuronide). According to the data of Levy et al. (1972) this difference can be expected to be less than a factor 2. Obviously this fact will make the approximation by one mixed non-linear pathway even better for salicylic acid than for ethanol. Furthermore, the relatively small difference between the constituting \( K_M \)'s leads to practically constant pooled parameters. Therefore, it is justified to treat salicylate kinetics on basis of equation 13.21. This conclusion cannot be
extended to the case of ethanol, since a difference of a factor 5 in $K_M$ values may lead to concentration-dependent pooled parameters (cf. also Sedman and Wagner, 1974). On the other hand it seems possible to obtain reliable estimates for the separate pharmacokinetic and metabolic parameters occurring in the equations 14.1. to 14.4 on basis of the concentration dependence of the fitted, pooled parameters. Requirements for this approach are firstly that the difference in individual $K_M$ values is a factor 5 or more and secondly that plasma curves after a wide range of doses can be measured. In this respect ethanol seems a promising drug, especially in animal experiments. These aspects are currently under investigation.

**APPENDIX I**

Integration of equation 14.1

\[
\frac{dC}{dt} = - \left[ \frac{f_1 K_{M_1}}{K_{M_1} + C} + \frac{f_2 K_{M_2}}{K_{M_2} + C} \right] \frac{C}{\tau_{el}} =
\]

\[
= - \left( \frac{f_1 K_{M_1} C + f_2 K_{M_2} C + K_{M_1} K_{M_2}}{(K_{M_1} + C) (K_{M_2} + C)} \right) \frac{C}{\tau_{el}} \tag{14.6}
\]

\[
\frac{(K_{M_1} + C) (K_{M_2} + C)}{(f_1 K_{M_1} C + f_2 K_{M_2} C + K_{M_1} K_{M_2}) C} \frac{dC}{dt} =
\]

\[
\left[ \frac{1}{C} + \frac{f_1 f_2 (K_{M_1} - K_{M_2})^2}{\{f_1 K_{M_1} + f_2 K_{M_2}\} \{K_{M_1} K_{M_2} + (f_1 K_{M_1} + f_2 K_{M_2}) C\}} \right. +
\]

\[
+ \frac{1}{f_1 K_{M_1} + f_2 K_{M_2}} \left. \right] \frac{dC}{dt} = - \frac{1}{\tau_{el}} \ dt \tag{14.7}
\]

Now integration is straightforward and with the boundary condition [$t=0$, $C=A$] the solution is equation 14.2.
APPENDIX II

Integration of equation 14.3

\[ \frac{dC}{dt} = - \left[ 1 - f_1 - f_2 + \frac{f_1 K_{M1}}{K_{M1} + C} + \frac{f_2 K_{M2}}{K_{M2} + C} \right] \frac{C}{\tau_{el}} \]

\[ = - \left[ \frac{K_{M1} K_{M2} + (1-f_2) K_{M1} C + (1-f_1) K_{M2} C + (1-f_1-f_2) C^2}{(K_{M1} + C)(K_{M2} + C)} \right] \frac{C}{\tau_{el}} \]

After rearrangement:

\[ \frac{(K_{M1} + C)(K_{M2} + C)}{(1-f_1-f_2) C} \left[ C^2 + \frac{(1-f_2) K_{M1} + (1-f_1) K_{M2}}{1-f_1-f_2} + \frac{K_{M1} K_{M2}}{1-f_1-f_2} \right] \frac{dC}{dt} = \]

\[ = - \frac{1}{\tau_{el}} \ dt \]

(14.8)

The quotient in the left side of equation 14.9 again can be split, so that we obtain:

\[ \left( \frac{1}{C} + \frac{a}{C-p} + \frac{b}{C-q} \right) dC = - \frac{1}{\tau_{el}} \ dt \]

(14.10)

where

\[ a = \frac{K_{M1} + K_{M2} + p + (1-f_1-f_2) q}{(1-f_1-f_2) (p-q)} \]

(14.11)

\[ b = \frac{K_{M1} + K_{M2} + q + (1-f_1-f_2) p}{(1-f_1-f_2) (q-p)} \]

(14.12)

and p and q are the roots of the quadratic equation between the brackets in the denominator of the left side of equation 14.9.

Integration of equation 14.10 leads simply to equation 14.4.
REFERENCES

CHAPTER 15 ACCUMULATION KINETICS IN CASE OF CAPACITY-LIMITED ELIMINATION

INTRODUCTION

Many drugs are administered to patients repeatedly over a certain period in a fixed schedule. One of the main objectives of applied pharmacokinetics is to enable physicians to predict the behaviour of the plasma concentration of drugs on chronic administration and to establish optimal dosage regimens. In combination with pharmacodynamic data, pharmacokinetics can give insight into the risk of too low, so subtherapeutic, dose levels as well as too high, possibly toxic levels. Especially for drugs with a small therapeutic index this information is extremely important. It is well-known for instance that effective antirheumatic action of salicylates requires plasma concentrations that are only slightly below toxic levels. This implies that in practice a patient often will be 'titrated' until the first signs of intoxication will become noticeable. As we have seen already salicylic acid is one of the compounds that exhibit capacity-limited elimination in a therapeutic dose range and study of its behaviour on chronic administration serves highly useful purposes, especially since the drug mostly is administered in repetitive regimens. The same holds true for phenytoin and ethanol; the former is used extensively as anticonvulsant-antiepileptic, the latter is an important source of abuse and since legal sanctions can be imposed, understanding of its behaviour in the body is highly important also for the proper authorities.

In this chapter the theoretical basis of drug accumulation in case of capacity-limited elimination will be discussed. Whenever possible, equations will be given that allow estimation of plateau levels, without extensive numerical calculation.

THEORY

When the elimination of a drug proceeds exclusively via linear, supply-limited mechanisms a very simple relationship exists between the average plateau concentration $\bar{C}_{pl}$, the dose D (in case of oral administration FD), the dosage interval $\Delta t$ and the overall clearance constant of the drug $k_{Cel}$:

$$\bar{C}_{pl} = \frac{FD}{\Delta t k_{Cel}}$$  (4.69)
This equation has been derived in chapter 4 and is valid, irrespective of
the number of compartments and the way of administration as long as
linear kinetics prevail (see also Van Rossum, 1968; Van Rossum and
Tomey, 1970). The average plateau concentration is directly proportional
to the 'dose flow' $\frac{D}{\Delta t}$ (e.g. in mg/hr) and inversely proportional to the
clearance constant. From this equation it is obvious that when the
clearance function is not a constant, but decreasing with increasing plasma
concentration, one should expect a disproportionate rise in plateau levels
with increasing dose flow. This implies that drugs with capacity-limited
elimination may easily be overdosed, when the dose is increased because of
a too low effectiveness. On the other hand, once toxic levels have been
reached a slight decrease in dose may cause subtherapeutic levels. Experimental
evidence for this will be discussed in chapter 16, with special reference to sodium salicylate. In the following paragraphs the mathematics of
accumulation in case of capacity-limited elimination are discussed. The
main problem here is that no explicit equations for the plasma concentra-
tion as a function of time are available. Therefore repeated oral adminis-
tration cannot be formulated analytically and the only way of calculating
plasma levels after chronic oral administration is by numerical integration
of the appropriate differential equations. There are, however, two other
cases where at least the plateau levels can be expressed in a general for-
numa, viz. repeated intravenous injections and continuous infusion of
drugs. In linear kinetics the average plateau concentration is independent of
the way of administration, but this cannot be assumed to be generally true
in capacity-limited kinetics. It is very well conceivable that the rate at
which a drug enters the body is determining the degree of saturation and
thereby the decrease of the clearance function and the increase of the
steady state levels. It is difficult to predict the exact influence of the rate
of entering, but it seems logical that repeated i.v. injections and continuous
infusion form the two extreme cases and that repeated oral administration
will lead to intermediate accumulation values. Therefore we give first the
two possible mathematical approaches and discuss the pitfalls afterwards.

I REPEATED I.V. INJECTIONS

Once a steady state has been reached the plasma concentration at the end
of any interval will be the same. Furthermore just after the injection the
plasma concentration will be higher than that at the end of the foregoing
interval to the amount of $D/V$. These obvious facts allow the following
analysis:

a. Capacity-limited elimination only.

From chapter 13 we have the equation for the plasma concentration:

$$\ln C = \ln A + \frac{A - C}{K_M} - \frac{t}{\tau_{el}}$$  \hspace{1cm} (13.13)

For plateau level calculations this equation can be rearranged to:

$$\ln C_{pl,\min} = \ln (C_{pl,\min} + \frac{D}{V}) + \frac{D}{VK_M} - \frac{\Delta t}{\tau_{el}}$$  \hspace{1cm} (15.1)

in which $C_{pl,\min} =$ the minimal plateau concentration (at the end of the dosage interval).

From equation 15.1 $C_{pl,\min}$ can be defined as:

$$C_{pl,\min} = \frac{D}{V \left[ e^{\left( \frac{\Delta t}{\tau_{el}} - \frac{D}{VK_M} \right)} - 1 \right]}$$  \hspace{1cm} (15.2)

In linear pharmacokinetics the same type of equation can be defined, the only difference being that the exponent in the denominator contains only $\Delta t/\tau_{el}$. Obviously equation 15.2 predicts much higher accumulation plateaus than in linear kinetics could be found. When $D/VK_M > \Delta t/\tau_{el}$, which means nothing else than $D/\Delta t > Q_m$ (= the metabolic capacity), no plateau at all will be obtained, but accumulation would go to infinity. This result was to be expected of course, since under those circumstances the dose per unit of time is higher than the maximum amount that can be eliminated during that time. The ratio between the ultimately reached plateau level and the level after a single dose is now determined not only by the dosage interval and $\tau_{el}$ but also strongly by the metabolic capacity. The maximal plateau concentration $C_{pl,\max}$ can easily be derived from equation 15.2 by making use of the relationship $C_{pl,\max} = C_{pl,\min} + D/V$. So:

$$C_{pl,\max} = \frac{D}{V \left[ 1 - e^{\left( \frac{D}{VK_M} - \frac{\Delta t}{\tau_{el}} \right)} \right]}$$  \hspace{1cm} (15.3)
b. Simultaneous supply- and capacity-limited elimination.
In general it will be unlikely that an extreme situation as depicted above prevails. Most probably part of the elimination process will be based on first-order processes. Therefore the use of equation 13.21 will be a more realistic approach:

\[
\ln C = \ln A + \frac{f}{1-f} \ln \frac{K_M + (1-f) A}{K_M + (1-f) C} - \frac{t}{\tau_{el}}
\]  

(13.21)

By analogous procedures as used above one can easily find expressions for \(C_{pl, \text{min}}\) and \(C_{pl, \text{max}}\). An important difference is, however, that no explicit equations for the plateau concentration can be formulated and that the resulting equations are not very convenient (see also Tsuchiya and Levy, 1972). It is interesting to note that in this case accumulation is restricted to an upper limit so that always a plateau will be reached (provided of course that \(f < 1\)). Another interesting feature is that at high dose and plasma levels the accumulation plateau ultimately is again directly proportional to the dose. This can easily be seen by assuming \(K_M\) in equation 13.21 to be negligibly small with respect to \((1-f) A\) and \((1-f) C\). Then equation 13.21 reduces to:

\[
\ln C = \ln A + \frac{f}{1-f} \ln \frac{A}{C} - \frac{t}{\tau_{el}}
\]  

(15.4)

For plateau level calculations this leads to:

\[
\ln C_{pl, \text{min}} = \ln \left( C_{pl, \text{min}} + \frac{D}{V} \right) + \frac{f}{1-f} \ln \frac{C_{pl, \text{min}} + \frac{D}{V}}{C_{pl, \text{min}}} - \frac{\Delta t}{\tau_{el}}
\]  

(15.5)

So that the following equation for \(C_{pl, \text{min}}\) is obtained:

\[
C_{pl, \text{min}} = \frac{D}{V \left[ e \left( 1-f \right) \Delta t / \tau_{el} - 1 \right]}
\]  

(15.6)

This equation is in fact the same as would be obtained in case of linear kinetics with a time constant for elimination of \(\tau_{el}/1-f\). Since in linear
pharmacokinetics the height of the average plateau concentration is
directly proportional to the time constant for elimination we can expect a
similar proportionality, but then with the ratio $\tau_{el}/1-f$ in case of partly
capacity-limited elimination. This clearly will give rise to much higher
plateau levels: the larger $f$, the higher the steady state level.

II CONTINUOUS INFUSION

The most convenient approach to pharmacokinetics of chronic adminis-
tration is to regard it as a continuous infusion, since then simple differenti-
tial equations can be defined, that can easily be integrated. In the plateau
situation the rate of infusion is equal to the rate of elimination (e.g. in
mg/hr) so that a simple equation results from which the plateau concen-
tration can be calculated. Applying this principle to a one compartment
model in its general form we obtain the following equation:

$$\frac{D}{\Delta t} = \dot{V}_{Cel} \cdot C_{pl}$$

(15.7)

where

$$\frac{D}{\Delta t} = \text{rate of infusion (e.g. mg/hr)}$$

$$\dot{V}_{Cel} = \text{clearance function (e.g. l/hr)}$$

$$C_{pl} = \text{plateau concentration (mg/l)}$$

In case of linear pharmacokinetics the plateau concentration calculated
this way is equal to the average plateau concentration that is reached after
repeated oral, i.v. or other administration. This is not so for drugs that are
(in part) eliminated by capacity-limited mechanisms. Then the concen-
tration $C_{pl}$ from equation 15.7 provides the lower limit of the expected
average plateau concentration after administration via another way.
Equation 15.7 can be specified for the two models that we consider in this
chapter:

a. Capacity-limited elimination only.
In this case the following relationship is obtained:

$$\frac{D}{\Delta t} = \frac{Q_m}{K_M + C_{pl}} \cdot C_{pl} = \frac{K_M k_{Cel}}{K_M + C_{pl}} \cdot C_{pl} = \frac{K_M V}{K_M + C_{pl}} \cdot C_{pl}$$

(15.8)
After rearrangement this leads to the following equation:

$$C_{pl} = \frac{K_M D/\Delta t}{\dot{Q}_m - D/\Delta t} = \frac{K_M D/\Delta t}{K_M k_{cel} - D/\Delta t} = \frac{K_M \tau_{el} D/\Delta t}{K_M V - \tau_{el} D/\Delta t}$$  \hspace{1cm} (15.9)

With the aid of this simple equation the average plateau concentration can be calculated very easily. As indicated already above no plateau will be reached when $D/\Delta t \geq Q_m$.

b. Simultaneous supply- and capacity-limited elimination.

In this case equation 15.7 may be specified as follows:

$$\frac{D}{\Delta t} = \left[1 - f + \frac{f K_M}{K_M + C_{pl}}\right] k_{cel} \hspace{1cm} C_{pl} = \left[1 - \frac{f C_{pl}}{K_M + C_{pl}}\right] k_{cel} C_{pl}$$  \hspace{1cm} (15.10)

Equation 15.10 can be rearranged to a quadratic equation in $C_{pl}$:

$$C_{pl}^2 + \left(\frac{K_M}{1-f} - \frac{D}{\Delta t (1-f) k_{cel}}\right) C_{pl} - \frac{D K_M}{\Delta t (1-f) k_{cel}} = 0$$  \hspace{1cm} (15.11)

This equation has two different roots, a positive one and a negative one. The relevant solution of course is the positive root. Equation 15.11 appears to provide a good approximation of the actual average plateau concentration that is reached also after chronic oral administration. The equation is especially useful since it can easily be solved without the need of computer facilities. The validity of this approach has been confirmed by comparison of the data obtained with values calculated by numerical methods (see below).

DISCUSSION

When drugs are administered for a long period of time, this most frequently occurs via the oral route. Since it is impossible to obtain explicit expressions for the course of the plasma concentration in case of capacity-limited elimination, one cannot formulate a general integrated
equation for the plasma concentration after chronic oral administration under these circumstances. Therefore, the plasma concentration has to be calculated by numerical integration of the appropriate differential equation, which is generally valid for a one compartment model, over the jth interval:

\[
\frac{dC}{dt} = \frac{D}{V\tau_a} \left( \frac{1 - e^{-j\Delta t/\tau_a}}{1 - e^{-\Delta t/\tau_a}} \right) e^{-t/\tau_a} - \frac{V_{Cel}}{V} C
\]  

where \( \tau_a \) = time constant for absorption of the oral dose
\( D \) = dose that is given in each interval
\( \Delta t \) = dosage interval
\( V \) = (apparent) volume of distribution
\( V_{Cel} \) = the (concentration-dependent) clearance function
\( t \) = time after last dose (0 \( \leq t \leq \Delta t \))

Figure 15.1 shows some examples of plasma curves simulated on basis of this equation. For comparative purposes the figure contains also the course of the plasma concentration after continuous infusion with the same dose flow as the oral curves. (It may be remarked that for the continuous infusion generally valid integrated equations for the plasma concentration as a function of time can be derived. The method of integration is similar to that given in the appendices to chapter 13 and 14.) The parameters used for the simulations in fig. 15.1 are in the range that we usually find for salicylate. From the figure it can be seen that irrespective of the dosage interval the plateau concentration ultimately reached is about the same when the total daily doses are equal. There is of course the normal difference in the amplitude of the fluctuations in the curve; the shorter the dosage interval the smaller the amplitude. A more specific consequence of capacity-limited elimination is the fact that in the steady state situation the amplitude of the oscillations is diminishing strongly with increasing plateau concentration, because the rate of elimination is then decreasing. Experimental examples of this phenomenon will be shown in the following chapter. For the curves of fig. 15.1 and for several others too, we calculated the average plasma concentration during the plateau situation. Table 15.1 gives a compilation of such values in comparison with the values calculated from equation 15.11. These data indicate that neither the dosage interval nor the absorption rate influence the average plateau concentration that ultimately is obtained, significantly. Furthermore the table illustrates convincingly the validity of the ‘infusion approach’: the values
Figure 15.1
Simulated course of the plasma concentration in case of combined supply- and capacity-limited elimination when a drug ($V = 8 \, l$, $\tau_{el} = 3.5 \, hr$, $f = 0.8$, $K_M = 25 \, mg/l$) is orally administered ($\tau_a = 1 \, hr$) or infused ($D/\Delta t = 125 \, mg/hr$). The dosage interval and consequently the degree of fluctuation is changed. See text for further explanation.

TABLE 15.1

Average plateau levels, $\bar{C}_{pl, num}$, numerically calculated on basis of equation 15.12 as compared with the values obtained by solving the quadratic equation 15.11 ($V = 8 \, l$, $\tau_{el} = 3.5 \, hr$, $f = 0.8$, $K_M = 25 \, mg/l$; these parameters are in the order of magnitude as found for salicylate). It may be noticed that the simple infusion approach yields accurate estimates of $\bar{C}_{pl}$ after chronic oral administration in these cases.

<table>
<thead>
<tr>
<th>$D$ (mg)</th>
<th>$\Delta t$ (hr)</th>
<th>$\bar{C}_{pl, num}$</th>
<th>$\bar{C}_{pl}$ (eq. 15.11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>8</td>
<td>185.5</td>
<td>185.3</td>
</tr>
<tr>
<td>750</td>
<td>6</td>
<td>185.4</td>
<td>185.3</td>
</tr>
<tr>
<td>500</td>
<td>4</td>
<td>185.3</td>
<td>185.3</td>
</tr>
<tr>
<td>2000</td>
<td>8</td>
<td>452.0</td>
<td>452.0</td>
</tr>
<tr>
<td>500</td>
<td>8</td>
<td>65.0</td>
<td>64.6</td>
</tr>
<tr>
<td>250</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
calculated from equation 15.11 are practically identical to those obtained by numerical integration of equation 15.12. This implies that for drugs with pharmacokinetic characteristics similar to salicylate we now have a simple and reliable method for calculation of plateau levels on chronic oral administration. Apart from the height of the plateau level, also the rate at which it is reached is important. Figure 15.2 gives an example of this from which it can be seen that the rise in the plasma concentration is somewhat

![Graph showing rate of accumulation in case of combined supply- and capacity-limited elimination.](image)

**Figure 15.2**
Rate of accumulation in case of combined supply- and capacity-limited elimination. Parameters: $V = 8 \text{ l}$, $\tau_{el} = 3.5 \text{ hr}$, $f = 0.8$, $K_M = 25 \text{ mg/l}$, $D/\Delta t = 125 \text{ mg/hr}$. $\Delta t$ is varied (from left to right: $\Delta t = 8, 6$ and $4 \text{ hr}$) and the right curve is based on continuous infusion. Note that the increase of the average plateau level is somewhat more rapid as the dosage interval increases, but that the ultimate plateau is the same.
faster for the longer dosage intervals. The reason for this is obvious: when a larger amount is administered at once the degree of saturation of the eliminating systems is higher. One might make use of this by administering a high first dose which can give rise to a practically instantaneous plateau level. This dosing procedure is especially effective for drugs with a largely capacity-limited elimination. On the other hand the saturation phenomenon leads to a retarded approach of the plateau level as compared to the case when only linear elimination occurs. In linear pharmacokinetics the plateau situation is reached when $j\Delta t \gg \tau_{el}$. When capacity-limited elimination prevails $\tau_{el}$ is increasing with increasing plasma levels. As a consequence the rate at which the ultimate plateau concentration is reached is smaller, although the accumulation quantitatively is much more pronounced than in linear kinetics.

In the figures 15.3 and 15.4 the average plateau level as a function of the dose and of $f$ (the fraction of the maximal total clearance proceeding via

![Figure 15.3](image)

*Figure 15.3*

Average plateau concentration as a function of the dose flow $D/\Delta t$, with varying contribution of the capacity-limited pathway ($f$). Note the much more than proportional increase of the plateau level when going from low to high doses. Obviously the degree of accumulation is strongly dependent on $f$ (see also fig. 15.4). Both the case with the highest degree of accumulation (repeated i.v. injections) and that with the lowest one (infusion) are plotted, but obviously these are hardly different for a drug with the parameters used for calculation ($V = 6 l$, $\tau_{el} = 3 hr$, $K_M = 25 mg/l$).
Figure 15.4
Average plateau concentration as a function of the contribution of the capacity-limited pathway to the overall clearance. Note the enormous increase of the average level when $f$ exceeds 0.5 (cf. legend to figure 15.3).

capacity-limited pathways) is depicted. The shaded areas represent the region in which the average plateau level in case of various absorption rates is lying. The upper limit is based on repeated intravenous injections, the lower limit on continuous infusion. Numerical calculations indicate that usually the infusion gives the best approximation of the oral case, unless absorption is extremely rapid. Figure 15.3 clearly illustrates that the increase in the plateau level with increasing dose is much more than directly proportional. At high dose levels, however, there is a proportional increase, as discussed above in this chapter. It is obvious that the fraction $f$ is a primary determinant for the degree of accumulation. This is confirmed
indeed by figure 15.4 which shows the enormous rise of the plateau level with increasing f at a constant dose. As a matter of fact the accumulation may proceed without an upper limit when f=1 (see above). Nevertheless, as long as f < 1 always a plateau will be reached. It should be noticed that in those cases where the plateau level is extremely changing with the dose, also the biological availability of the drug on oral administration has profound influence on the plateau characteristics.

In conclusion it appears that the simple ‘infusion approach’ outlined in this chapter provides very useful information concerning the accumulation of drugs that are eliminated (in part) by capacity-limited pathways. There are several examples of papers appearing in the literature, where the authors failed in describing adequately the steady state levels observed. A typical example can be found in the work of Lund et al. (1974) on the pharmacokinetics of phenytoin after multiple doses. When the procedures outlined in this chapter are applied to their data it appears to be easy to understand the observations and to predict plateau levels of phenytoin after chronic administration or infusion. The same holds true for studies on ethanol accumulation (Forney and Hughes, 1963; cf. also Levy, 1975).

REFERENCES

CHAPTER 16 PHARMACOKINETICS OF SALICYlates IN MAN

A. INTRODUCTION

In the beginning of the 19th century there was an industrious search for quinine substitutes. Many bark preparations with a bitter taste were tested. The white willow (salix alba) appeared to be a promising source of antipyretic preparations and in 1827 from the bark of this tree salicin (glycoside of salicylalcohol) was isolated by Leroux. Salicin was the starting material for the synthesis of salicylic acid by Piria in 1838. Salicylic acid became available in large amounts when Kolbe developed his famous synthetic route (1860). The antipyretic effectiveness of salicylic acid was soon established, but its oral use had serious drawbacks because of its bad, bitter taste but especially because of gastro-intestinal complaints. The free acid is very irritating to the mucosa of the gastro-intestinal tract and in high concentrations it even has a keratolytic effect. For the latter purpose it is applied externally up to today. An important improvement was the introduction of sodium salicylate by Buss in 1875. Sodium salicylate appeared to be a useful therapeutic for the treatment of rheumatic fever and it was successfully applied to other rheumatic diseases as well. Many other derivatives of salicylic acid have been prepared afterwards with the objective to find a more suitable and more convenient administration form of the pharmacologically active salicylate ion. Here we only mention one of these, viz. acetylsalicylic acid, introduced by Dreser in the nineties of the last century. Acetylsalicylic acid was found to have about equal antiinflammatory and antipyretic action as sodium salicylate but a much better general analgesic action. Ever since their development the salicylates, especially sodium salicylate and acetylsalicylic acid, have been the drugs of choice for the treatment of inflammatory disorders. Furthermore acetylsalicylic acid (aspirin, which in some countries is still a trade name) is the active substance in numerous antipyretic and analgesic preparations, many of which are available without prescription. The salicylates have been the subject of an enormous number of studies. Many relevant aspects have been reviewed in comprehensive monographs (Gross and Greenberg, 1948; Dixon et al., 1963; Smith and Smith, 1966). Since the appearance of these books the flow of information is still increasing. Side-effects and toxicity have been comprehensively reviewed by Prescott (1972). In this introduction shortly some important data on the metabolism and the pharmacokinetics of salicylic and acetylsalicylic acid will be reviewed that
are significant for interpreting the results of our own investigations.

Acetylsalicylic acid is rapidly deacetylated in man to salicylic acid, the half life is about 20 minutes (Rowland et al., 1967, Rowland and Riegelman, 1968, Rowland et al., 1972). Although acetylsalicylic acid is unstable in aqueous solution it was established that on oral administration the drug itself is absorbed, leading to substantial plasma levels for the first hour after administration (see also Mandel et al., 1954, Leonards, 1962). The rapid deacetylation of acetylsalicylic acid implies that only formulations that are very fast absorbed can be considered when the superiority as an analgesic of acetylsalicylic acid over salicylic acid is aimed at. Both acetylsalicylic acid and salicylic acid have a small volume of distribution (≤ 10 l). Whereas the elimination of acetylsalicylic acid appears to proceed exclusively by deacetylation, salicylic acid shows a more complex pattern. The elimination pathways are summarized in figure 16.1, based on literature data (Levy et al. 1969, 1972, Tsuchiya and Levy, 1971, Bedford et al., 1965, Boreham and Martin, 1969, Schachter and Manis, 1958,)

*Capacity limited in therapeutic dose range

Figure 16.1
Pathways of elimination of salicylic acid in man.
Many pharmacokinetic studies have been performed with salicylic acid in man and a large range of plasma half-lives have been reported (e.g. 6 hrs: Brodie et al., 1959; 19 hrs: Swintosky, 1956; 3.5–4.5 hrs: Rowland and Riegelman, 1968). In several of the references mentioned the authors indicated that the kinetic behaviour of salicylate was apparently dose-dependent, in the sense that the rate of elimination was decreasing with increasing dose. As the crown on very nice thorough analyses of urinary excretion patterns of the metabolites of salicylic acid, especially by Levy and co-workers, in 1972 the effective characterization of salicylate elimination was accomplished (Levy et al., 1972). Two of the metabolic pathways appear to be capacity-limited in a therapeutic dose range, viz. the formation of salicyluric acid and of salicyl phenolic glucuronide. As demonstrated in chapter 13 (van Ginneken et al., 1974) also the profile of the plasma curve can be adequately described on basis of Michaelis-Menten kinetics. As far as the renal excretion of unchanged salicylate is concerned, we present evidence in this chapter that non-linear mechanisms are involved. In fact already in 1955 Gutman et al. indicated that tubular secretion must be assumed to contribute to the excretion of salicylate. Salicylic acid may be subject to other non-linear processes as well. Evidence has been given for instance for non-linear disposition of salicylic acid in rat brain (Gonzalez et al., 1975). Autoradiographic studies in mice, however, have shown that only very small amounts of salicylate penetrate into brain (van Ginneken and van Rossum, unpublished results). Salicylate crosses readily the placenta barrier, its concentration in neonatal plasma appeared to be even higher than in maternal plasma (see for a discussion of this phenomenon Garretson et al., 1975 and Levy et al., 1975). Salicylates are mostly applied in case of joint disease and therefore it is interesting to note that they appear quite rapidly in the joint fluid in a maximum concentration about 2/3 of the plasma concentration, independently of the disease state, which on the other hand does influence the rate of appearance (Soren, 1975).

Some authors suggested that the kidney might be responsible for an important part of salicylate metabolism, especially salicyluric acid formation (von Lehmann et al., 1973). However, the finding of Lowenthal et al. (1974), that anephric patients show a normal rate of formation of salicyluric acid and other metabolites seems to rule out the possibility of renal metabolism.

The present investigations were performed in order to test the several aspects of capacity-limited elimination as outlined in the chapters 13–15, both on single and multiple dosing. The main goal was to obtain quanti-
tative insight in the accumulation characteristics of salicylate and the factors that are primary determinants for this. It needs not to be said that such information is extremely valuable for establishing and interpreting dosage regimens in clinical practice. As will be discussed, plateau levels of salicylate are highly sensitive to changes in dose (or biological availability), but also to changes in the nature of the clearance function. In this respect the renal clearance of unchanged salicylate may be of utter importance. Therefore the excretion by the kidneys was studied in some detail. Also some biopharmaceutical aspects are included. These are specifically meaningful in two ways. First it has been mentioned already that for general analgesic aims acetylsalicylic acid should be dispensed in a dosage form that is very rapidly absorbed. This is the more so since acetylsalicylic acid has much more side-effects than sodium salicylate, so that only when appropriate use is made of its higher effectiveness, acetylsalicylic acid is to be preferred over sodium salicylate. Secondly, other biopharmaceutic characteristics may be desired for long term therapy of inflammatory conditions. In this case especially the height and the constantness of the plasma concentration are important features, rather than a shortlived peakconcentration. It will be shown indeed that drug formulations, that are hardly useful for single dose administration, may be very suitable for sustaining constant plasma levels.

B. MATERIALS AND METHODS

Drugs
The following dosage forms have been applied in this study:
Sodium salicylate aqueous solution, containing sodium salicylate and sodium bicarbonate in a weight ratio 2:1; Enterosalicyl® tablets (Sarein, France), containing 500 mg sodium salicylate (~431 mg salicylic acid) in an enteric coated form; Rhonal® tablets (Specia, Paris, France), containing 500 mg acetylsalicylic acid (~385 mg salicylic acid) in the form of coated microcrystals; Ascal® powder (ACF Chemiefarma, Maarssen, The Netherlands), containing calcium acetylsalicylate corresponding with 500 mg acetylsalicylic acid; Salipyrin (phenazone salicylate), an equimolecular compound of phenazone and salicylic acid, administered as an aqueous suspension; Sodium benzoate solution containing sodium benzoate and sodium bicarbonate in a weight ratio 2:1; Benemid® tablets (MSD, Westpoint Pa, USA) containing 500 mg probenecid; D-glucuronolactone aqueous solution; Diuramon® tablets (ACF Chemiefarma, Maarssen, The Netherlands) containing 400 mg ammoniumchloride in enteric coated
form, Sodium bicarbonate in powder form

Sodium salicylate, sodium benzoate and phenazone salicylate (Salipyrin) were obtained from OPG, Utrecht, The Netherlands, D-glucuronolactone from Sigma, St. Louis, Mo, USA.

**Drug administration**
All participants in this study were young, male healthy volunteers (age 20—25 years). Furthermore 4 of the subjects participating in the investigations on repeated administration were patients who were taking salicylates chronically because of rheumatic disease (patients K, Z, J and T).

In the single dose experiments the subjects were fasting overnight prior to and for an additional 2 hrs after drug intake. Four subjects received oral doses of sodium salicylate solution, Ascal®, Rhonal® and Enterosalicyl® in a crossover design, with intervals of one week. Four other volunteers took Salipyrin (aqueous suspension) and equimolecular doses of sodium salicylate (solution) and phenazone (solution) at biweekly intervals. In the experiments on chronic administration the subjects took a dose 3 times daily and in one case (subject T) 4 times daily for periods of at least one week. In the investigations of kinetic interactions the subjects took a single dose of sodium salicylate in solution (usually 750 mg) and several doses of the substance that was assumed to affect the kinetic behaviour of salicylate. The doses were planned to yield a constant plasma level of these substances. In case of benzoate and D-glucuronolactone pilot experiments were performed in order to obtain insight into the pharmacokinetics of these substances. For probenecid sufficient data are available in literature (Dayton et al., 1963).

In all experiments bloodsamples were taken from a fore arm vein, at regular times after drug administration. In most cases time and total volume of every aliquot of urine voided were registered and samples were collected for analysis.

**Preparation of samples and spectrophotofluorometric analysis**
As described in chapter 2. All concentrations were determined in terms of salicylic acid.

* The kind co-operation of Prof Dr C L H Majoor (Head of the department of Internal Medicine, University of Nijmegen) and Dr F W J Gribnau (departments of Internal Medicine and Pharmacology) is gratefully acknowledged in this connection.
C. PLASMA CURVES AFTER SINGLE DOSE

It has been discussed already in chapter 13 that the pharmacokinetic behaviour of salicylate can be described on basis of combined capacity-limited and supply-limited elimination. This implies that the profile of the plasma curve for salicylate (assuming a one-compartment model) is fully defined by four fundamental kinetic parameters: the volume of distribution ($V$), the maximally obtainable total body clearance ($k_{Cel}$), the apparent Michaelis-Menten constant ($K_M$) and the fraction that is eliminated by the saturable pathway ($f$). Figure 16.2 gives an example of a plasma curve together with the renal excretion of salicylate (see also fig. 13.5) and table 16.1 shows these parameters as they were obtained in several experiments. The values have been obtained by application of the computerfit program Farmfit. All data have been handled according to a one-compartment model with intravenous administration. However, sodium salicylate was always given orally, but since the absorption was very rapid as compared to the elimination the i.v. model gives a good approximation. It should be remarked that the curves will deviate from what is found after i.v. administration since distribution is concealed under the absorption phase.

TABLE 16.1
Pharmacokinetic parameters for salicylic acid obtained after oral administration of a single dose of sodium salicylate in aqueous solution

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose (mg)</th>
<th>V/F (l)</th>
<th>$K_M$ (mg/l)</th>
<th>f</th>
<th>$\tau_{el}$ (hr)</th>
<th>$k_{Cel}$ (l/hr)</th>
<th>$Q_m$ (mg/hr)</th>
<th>$Q_r$ (% dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>750</td>
<td>61</td>
<td>38</td>
<td>0.77</td>
<td>2.9</td>
<td>2.1</td>
<td>61</td>
<td>9.4</td>
</tr>
<tr>
<td>HA</td>
<td>750</td>
<td>86</td>
<td>33</td>
<td>0.85</td>
<td>3.1</td>
<td>2.8</td>
<td>79</td>
<td>10</td>
</tr>
<tr>
<td>JBo</td>
<td>800</td>
<td>7.9</td>
<td>30</td>
<td>0.89</td>
<td>2.3</td>
<td>3.4</td>
<td>91</td>
<td>12.2</td>
</tr>
<tr>
<td>BC</td>
<td>750</td>
<td>7.7</td>
<td>37</td>
<td>0.72</td>
<td>4.5</td>
<td>1.7</td>
<td>45</td>
<td>0.4</td>
</tr>
<tr>
<td>AL</td>
<td>750</td>
<td>7.8</td>
<td>31</td>
<td>0.84</td>
<td>3.1</td>
<td>2.5</td>
<td>65</td>
<td>5.6</td>
</tr>
<tr>
<td>JM</td>
<td>750</td>
<td>8.3</td>
<td>56</td>
<td>0.78</td>
<td>2.9</td>
<td>2.9</td>
<td>127</td>
<td>5.3</td>
</tr>
<tr>
<td>WR</td>
<td>750</td>
<td>8.3</td>
<td>56</td>
<td>0.78</td>
<td>2.9</td>
<td>2.9</td>
<td>127</td>
<td>5.3</td>
</tr>
<tr>
<td>HS</td>
<td>770</td>
<td>6.0</td>
<td>26</td>
<td>0.79</td>
<td>2.8</td>
<td>2.1</td>
<td>44</td>
<td>15.1</td>
</tr>
<tr>
<td>HV</td>
<td>1150</td>
<td>6.0</td>
<td>27</td>
<td>0.91</td>
<td>2.5</td>
<td>2.4</td>
<td>59</td>
<td>15.1</td>
</tr>
<tr>
<td>JZ</td>
<td>750</td>
<td>6.5</td>
<td>34</td>
<td>0.80</td>
<td>3.0</td>
<td>2.2</td>
<td>59</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>6.6</td>
<td>31</td>
<td>0.78</td>
<td>3.1</td>
<td>2.1</td>
<td>51</td>
<td>8.9</td>
</tr>
</tbody>
</table>

1. all subjects male, age 21-26 yr, body weight 64-82 kg
2. dose expressed as free salicylic acid
3. $Q_r$ = amount of salicylic acid excreted in urine unchanged

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Figure 16.2
Plasma curve and renal excretion of salicylic acid after oral administration of a dose of 800 mg (in the form of a sodium salicylate solution). Note the non-linear decay of the plasma concentration.

Volume of distribution
The volume of distribution cannot be determined absolutely after oral administration, since the biological availability of the drug is not known. Comparison of our data with those of Rowland and Riegelman (1968) after i.v. administration suggests that the availability of the salicylate in aqueous solution must be nearly complete. Also comparison of the solution with other formulations (see below) indicates the highest biological availability for the solution. The absorption rate of salicylate from the aqueous solution can be extremely high. Time constants for absorption have been estimated graphically and they appear to be in the range from 0.5 to 10 minutes, in most cases not exceeding 3 minutes. The first blood sample usually was taken after 10 minutes and in practically all experiments at that time already maximal plasma levels had been reached. Such high absorption rates have been discussed already in chapter 10C. Apart
from the factors indicated there it should be taken into account that the absorption of salicylate may start in the stomach already. Nevertheless the major part will be absorbed from the small intestine, especially when as in our study the drug is administered in solution and on an empty stomach. The volume of distribution of salicylic acid is small. The table shows values that all are smaller than 10.1, which is comparable to the values found for ibuprofen (chapter 5) and alclofenac (chapter 8). This small volume of distribution is related to the physicochemical properties of the drug: salicylic acid is bound to plasma proteins for about 70% at total plasma levels of 50 mg/l (Kucera and Bullock, 1969). This is substantially less than found for ibuprofen, but on the other hand the lipid solubility of salicylic acid is lower than that of ibuprofen. These two factors seem to counterbalance each other so that the same distribution volumes are found for both drugs. As compared to alclofenac, salicylic acid is more lipophilic and probably more protein bound, so the situation is just the other way around. Salicylic acid takes an intermediate position among the three drugs that all have approximately the same volume of distribution. In a physiological sense the distribution volume will represent primarily the extracellular fluid. Indirect evidence for this may be found in the fact that after single dose experiments the concentration of salicylate in the red blood cells appeared to be only 1—2% of the corresponding plasma concentration. Interestingly in vitro much higher penetration into erythrocytes has been reported (Schanker et al., 1964). The reason for this discrepancy is not clear, especially not since in vivo the concentration in the erythrocytes varied proportionally with the plasma concentration, indicating a rapidly established distribution equilibrium. Nevertheless, the fact that such low erythrocyte levels are found suggests that also in other cells the concentration will be much lower than the plasma concentration. Whole body autoradiography in mice yielded results that are fully in line with this expectation (van Ginneken and van Rossum, unpublished data).

The apparent Michaelis-Menten constant $K_M$

$K_M$ is the parameter that primarily determines whether or not the slope of the semilogarithmic plasma curve changes with the dose. As long as the plasma concentration is small as compared to $K_M$ no signs of capacity-limitation will be visible and a slope will be obtained which corresponds to the case that no saturation at all occurs and the clearance is maximal. In view of the $K_M$ values reported in table 16.1 the ultimate time constant for elimination may only be determined with reasonable accuracy when sufficient concentration data are measured below a limit of about 20 mg/l
and even then computerfit will be necessary since by graphical procedures the endslope will always be underestimated. The $K_M$ used in the description of salicylate elimination is a purely operational parameter and not a true enzymatic constant. It has been discussed before that at least two saturable metabolic pathways are involved, but furthermore the renal excretion exhibits clearly non-linear features (see below). This implies that the $K_M$ in this context is a complex parameter made up by at least three elementary constants and their respective weights. Fortunately the three components have values that do not differ too much so that the combined parameter for all practical purposes may be regarded as a dose-independent constant (see also discussion in chapter 14). This is substantiated by the experiments in which different doses have been administered to the same volunteer: the $K_M$ values obtained are in no case significantly different. It is important to note that the $K_M$ values in table 16.1 are calculated on basis of salicylic acid concentrations. Of course other values would be obtained when the parameters were expressed on basis of another molecular species (for instance sodium salicylate), the difference being a constant factor, correcting for differences in molecular weight. Since $K_M$ is derived from concentration data only, its estimation is independent of the volume of distribution (and the biological availability). The same holds true for the estimation of $f$, the fraction of the maximal total body clearance proceeding via saturable pathways.

The fraction $f$
Under normal circumstances $f$ appears to be in the order of 0.80. Large variations may occur, however, in the estimation of this parameter. Since such variations have profound influences on the profile of the plasma curve but especially on the height of the plasma levels reached after chronic administration, it is of extreme importance to look after the factors that may alter $f$. From figure 16.1 it can been seen that two metabolic pathways are saturable at usual therapeutic dose levels, viz. formation of salicyluric acid and of salicyl phenolic glucuronide. It is conceivable that part of the variability of $f$ is due to differences in the capacity of these two mechanisms. This may account for some interindividual variation but definitely not for the intraindividual variation. Furthermore in many cases $f$ tends to be higher than the maximal contribution of these metabolic pathways. As indicated already it is very likely that $f$ comprises also part of the renal excretion. Now the renal excretion of salicylate is a very complicated process (see below) but we can be sure that the fraction of the total amount appearing in urine that is excreted by a linear process is

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highly dependent on urinary pH. Acidification of urine leads to a clear
decrease of the contribution of linear excretion and to an equally clear
increase in f, whereas alkalinization has the opposite effect. Actually these
changes may be drastic enough to explain most of the intraindividual
variations in f that are experimentally encountered. Unfortunately for the
moment it is impossible to incorporate these factors into a generally
applicable model for description of salicylate kinetics under variable
urinary pH. One of the objectives of our current investigations is to find a
relationship between f and urinary pH, that combines easy accessibility
with sufficient predicting value. The enormous advantage of such a rela-
tionship will become clear in the discussion of plateau levels after chronic
administration.

Parameters describing the ultimate, linear elimination
Table 16.1 contains also values for \( \tau_{el} \) and \( k_{Ce1}/F \). These parameters
have the same meaning as in linear pharmacokinetics (section II). In case
of salicylate they refer to the linear phase that is reached when the plasma
concentration is sufficiently small with respect to the operational \( K_M \).
Roughly speaking these parameters are the sole determinants of the plasma
decay curve when doses of less than about 500 mg acetylsalicylic acid or
sodium salicylate are given. It may be noted that \( \tau_{el} \) (and \( t_{1/2} \), which is
0.69\( \tau_{el} \)) are rather small. The halflife as a rule is somewhere around
2.5 hrs, which is smaller than values that have been published in literature
(see introduction). The explanation is obvious: when concentration data
of salicylate are graphically analysed according to linear pharmacokinetics
the halflife will always be overestimated, unless the concentration is
followed for long times and to very low levels; only computerized analysis
on basis of the appropriate non-linear model can yield adequate estimates
of the ‘true’ halflife. The (maximally attainable) clearance constant \( k_{Ce1} \) is
in the same order of magnitude as found for ibuprofen and alclofenac
(chapter 5 and 8). Only the phenomenon of saturable metabolism makes
the clearance of salicylate totally different from that of these drugs.

The capacity of the saturable pathways \( \dot{Q}_m \)
When dealing with one capacity-limited pathway of elimination, the
relevance of \( \dot{Q}_m \) is straightforward. Then it represents the maximal
amount of drug that can be eliminated via that pathway. As a matter of
fact the term ‘capacity-limited’ is derived from the phenomenon that in
such a case at very high plasma concentrations a constant amount of drug
is eliminated per unit of time, as determined by the capacity of the system
(\(Q_m\)). A simple, clearcut formula gives the relationship between \(Q_m\) and other parameters:

\[
\dot{Q}_m = f \cdot K_M \cdot k_{\text{Cel}}
\]  

(16.1)

As argued before (chapter 13) in our fitting procedure we use equations from which \(Q_m\) is eliminated by use of this equation, but of course it can easily be calculated from the fitted parameters. In case of salicylate \(K_M\) is some kind of weighted average of individual Michaelis-Menten constants and \(f\) is constituted by at least three different processes. Therefore a value for \(Q_m\) calculated according to equation 16.1 (as given in table 16.1) cannot be interpreted in terms of single processes but is only meaningful in as far as it may give an impression of the total amount of drug that can be eliminated by all saturable processes together.

D. RENAL EXCRETION

The renal excretion of salicylate is strongly dependent on the pH of the urine (Smith et al., 1946; Williams and Leonards, 1948; Hoffman and Nobe, 1950; Smith and Smith, 1966; Levy and Leonards, 1971). The typical pictures that are obtained by plotting the apparent renal clearance constant versus the urinary pH are illustrated in figure 16.3. Over the pH traject from 5.5 to 7 the renal clearance constant shows an increase of as much as a factor 100. When compared with the total body clearance constant of salicylate this implies that at pH 5.5 about one percent of the clearance proceeds by renal excretion whereas at high urinary pH this percentage may rise up to 30. This, however, is a simplification of the actual behaviour since part of the elimination occurs by saturable ways. As a matter of fact figure 16.4 shows that the renal clearance itself is a complicated concentration-dependent process. This figure gives the relationship between the renal excretion rate of salicylate and the average plasma concentration over the interval during which the excretion rate is estimated, in one and the same individual. To avoid pH influences as far as possible only excretion rates at a urine pH between 5.5 and 6.0 are plotted, since in this range the pH dependence of the excretion is rather small. Obviously the graph exhibits three distinct phases, which may easily be explained by assuming three processes to correspond with these phases, viz. tubular secretion, active tubular reabsorption and glomerular filtration
(combined with passive, pH-dependent backdiffusion). Further it has to be assumed that salicylate has a higher affinity (so a lower $K_T$) for the secretion mechanism than for the reabsorption mechanism, and that the capacity of the active reabsorption exceeds that of the secretion substantially. Under these assumptions the first rapid increase in excretion rate corresponds primarily with tubular secretion which is soon saturated. The drop in the curve represents the active reabsorption mechanism which becomes predominant especially after the secretion has become saturated. Ultimately, when both secretion and active reabsorption are saturated, further increase in plasma concentration leads to a proportionally increase in renal excretion rate. The slope of the resulting straight line then is determined exclusively by the linear part of the renal excretion process, so the sum of filtration and passive backdiffusion. From the data of figure 16.4 it can be derived that subject JB shows a linear renal clearance constant (filtration and backdiffusion) of about 0.6 ml/min at urine pH values of 5.5—6.0. This falls in the range that is normally encountered. The non-linear processes cannot be analysed quantitatively on basis of this type of plots. We can only estimate the difference in the capacity of the two
non-linear mechanisms. It appears that the capacity of the active reabsorption exceeds that of the secretion to the amount of 50 µg/min (3 mg/hr). It is interesting to note that the possible explanation given here coincides with the well-known paradox effect of salicylates on uric acid excretion.

![Graph showing renal excretion rate vs. plasma concentration](image)

**Figure 16.4**

Renal excretion rate (at urine pH 5.5–6) of salicylic acid as a function of the corresponding average plasma concentration. As discussed in the text, the curve indicates the occurrence of tubular secretion as well as active reabsorption. The curve can be analysed to get an impression of the relative contributions of filtration, combined with passive backdiffusion (GF) and of tubular secretion, combined with active reabsorption (TS + AR). Obviously, the capacity of the active reabsorption mechanism exceeds that of the secretion mechanism.

This paradox effect implies the phenomenon that salicylates in low doses inhibit uric acid excretion whereas high doses have the opposite effect. An attractive assumption to account for this paradox is that low doses of salicylate inhibit the tubular secretion of uric acid, whereas only at high doses the active reabsorption of uric acid from the tubular urine may be
inhibited to such a degree that the net result is an increase in the amount of uric acid excreted. The type of plots as depicted in figure 16.4 seems to be very useful for interpreting renal clearance mechanisms and the explanation we gave for the course of the graph is supported by a lot of previous research. Especially the elegant work of Gutman et al. (1955) on the mechanism of renal excretion of salicylate provides strong evidence in favour of the complicated excretion patterns discussed here. Gutman could not establish the occurrence of active reabsorption beside passive backdiffusion from the renal tubuli. In view of our data, however, it seems reasonable to assume the existence of such a mechanism. The picture of figure 16.4 is a very general one, at least when urinary pH is kept low (pH < 6.5). Similar patterns were obtained in all subjects in whom we

![Graph](image)

**Figure 16.5**

*Renal excretion rate of salicylic acid as a function of plasma concentration in the same subject as in figure 16.4, but at higher urinary pH (6.5–7). It may be noticed that the relationship as seen in figure 16.4 is not seen here. See text for a discussion of this difference.*
were able to measure salicylate levels and corresponding excretion rates over wide ranges of plasma concentration. Difficulties arise, however, when higher urinary pH values are involved. At increasing pH the passive backdiffusion of salicylate decreases strongly (figure 16.3), so that the linear part of the excretion process becomes increasingly important. On the other hand it is likely that the active mechanisms that contribute to the overall excretion are not or much less dependent on the pH of the urine. Then one might expect that the increase in the efficiency of the filtration process conceals the occurrence of more specific mechanisms. This is found indeed as exemplified in figure 16.5. The data in this figure are obtained in the same subject as the data in figure 16.4, but now the urine pH varies from 6.5 to 7.0. Clearly the data points are much more scattered, which is presumably caused by the fact that in this pH interval the pH dependence is so strong already that no uniform filtration efficiency is met anymore. The large variability of the renal clearance of salicylate under the influence of urinary pH suggests that this might be reflected in the plasma curve. Therefore we performed some investigations in which the volunteers ingested ammoniumchloride or sodiumbicarbonate in order to produce acid and alkaline urine respectively. The intake of the salts was started on the morning of the day prior to the day at which the experiments began and was continued throughout the course of the

<table>
<thead>
<tr>
<th>Subject</th>
<th>Treatment</th>
<th>V/F (l)</th>
<th>$K_M$ (mg/l)</th>
<th>$\tau_{el}$ (hr)</th>
<th>$Q_r^2$ (% dose)</th>
<th>$\text{pH}_{ur,av}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN</td>
<td>NaHCO₃</td>
<td>9.0</td>
<td>48</td>
<td>0.95</td>
<td>2.9</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>NH₄Cl</td>
<td>9.2</td>
<td>56</td>
<td>1.00</td>
<td>3.3</td>
<td>0.5</td>
</tr>
<tr>
<td>LS</td>
<td>NaHCO₃</td>
<td>7.8</td>
<td>45</td>
<td>0.92</td>
<td>3.2</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>NH₄Cl</td>
<td>8.1</td>
<td>45</td>
<td>1.00</td>
<td>3.4</td>
<td>4.3</td>
</tr>
<tr>
<td>RS</td>
<td>NaHCO₃</td>
<td>6.8</td>
<td>28</td>
<td>0.78</td>
<td>3.5</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>NH₄Cl</td>
<td>7.6</td>
<td>25</td>
<td>0.92</td>
<td>4.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

1. $\tau_{el}$ refers to the ultimate straight part of the plasma curve
2. $Q_r$ = amount of salicylic acid that is excreted in urine unchanged
3. $\text{pH}_{ur,av}$ = weighted average of the pH of all urine samples produced during each experiment
experiment. The dose was 3 and 6 g daily for sodium bicarbonate and ammonium chloride respectively, divided in 6 fractions. Figure 16.6 shows an example of the plasma curves obtained and table 16.2 summarizes the relevant data and fitted parameters. Clearly the concurrent administration of ammonium chloride leads to a slower elimination of salicylate from plasma than the bicarbonate treatment. This was to be expected on basis of the influence on renal excretion of unchanged salicylate. Table 16.2 indicates that the fraction of the dose that is excreted in urine is much higher after sodium bicarbonate than after ammonium chloride. Further it should be noted that the apparent \( K_M \) values are not different in the two sets of experiments, whereas \( f \) tends to increase on ammonium chloride treatment. Also this is not unexpected: \( K_M \) should be independent of passive backdiffusion mechanisms, and the fraction that is eliminated by capacity-limited pathways should increase when the linear renal clearance decreases. The differences in \( f \) are not statistically significant because of

![sodium salicylate profile](image)

**Figure 16.6**

Influence of alkalinization or acidification of the urine on the profile of the plasma curve of salicylic acid. Note the lower rate of elimination, when \( NH_4 Cl \) is ingested. See text for further details. See also table 16.2.
the relatively large error in this parameter, but they are consistent and therefore they seem to point at real, important variations in elimination mechanisms. Although the shift in f at first sight may be rather small, it must be stressed that such differences have profound influences on the accumulation characteristics (see for instance figure 15.4).

E. PHARMACOKINETIC INTERACTIONS

The fact that the elimination process of salicylates is well-understood in detail suggests several interaction possibilities, both on the level of hepatic metabolism and on the level of renal excretion. In the following some investigations in this field and their possible implications are discussed. First it should be defined what kind of kinetic drug interactions may be expected. The most understandable approach is to return to the clearance function for a single metabolic pathway of elimination

\[ \dot{V}_{Cel} = \frac{\dot{Q}_m}{K_M + C} \]  

(13.8)

(The same formula is applicable for tubular secretion processes)

The equation indicates that two parameters may be modified by other drugs. The first is the metabolic capacity (or the tubular transport maximum) of the pathway. This metabolic capacity may be increased (with enzyme inducers such as phenobarbital etc.) or decreased (by irreversible enzyme inhibitors, uncouplers of metabolic chains, substances that cause tissue damage etc.). Also in various pathological situations the metabolic capacity will be subject to alteration. The second parameter, to which the further discussion will be restricted, is the apparent Michaelis-Menten constant \( K_M \). Whereas non-competitive interactions will influence primarily \( \dot{Q}_m \), all competitive interactions (between substances that are metabolised or transported by the same system) are reflected in \( K_M \). When a second substance is introduced at a concentration \( I \) and with an apparent Michaelis-Menten constant \( K_I \) (I from ‘inhibitor’), equation 13.8 obviously becomes:

\[ \dot{V}_{Cel} = \frac{\dot{Q}_m}{K_M \left(1 + \frac{I}{K_I}\right) + C} \]  

(16.2)

This implies that any competitive inhibitor will decrease the clearance function, provided that its concentration is not very small with respect to its corresponding \( K_I \). Clearly this effect is not limited to cases where
capacity-limited elimination occurs. Also when \( C \ll K_M \) the clearance, which then is a concentration-independent constant, is lowered by the presence of an inhibitor. Actually possible signs of saturation will become less pronounced by the inhibitor, which causes an increased apparent \( K_M \) value. So, whether kinetic interaction via \( K_M \) takes place or not and to what degree, is only dependent on the ratio \( I/K_I \) of the inhibitor and not on the enzymatic parameters of the drug itself. In the light of these considerations three different types of interaction were investigated.

a. Benzoic acid
Benzoic acid is known to be metabolised almost exclusively by conjugation with glycine, leading to the metabolite hippuric acid, which is extremely rapidly excreted in urine (Quick, 1931; Williams, 1959; Wu and Elliot, 1961; Schachter, 1956; Levy, 1965). On basis of detailed analysis of renal excretion data the expectation that benzoic acid would inhibit competitively the formation of salicyluric acid, the main metabolite of salicylic acid, has been confirmed (Levy and Amsel, 1966; Amsel and Levy, 1969). In this

![Figure 16.7](image-url)

*Plasma curves after sodium salicylate alone and in combination with sodium benzoate. Benzoate appears to inhibit the elimination of salicylate. See text for details and table 16.3.*

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report we demonstrate the effect of benzoic acid on the plasma curve of salicylic acid. The fact that benzoic acid seems to be a tool for affecting specifically one of the pathways of salicylate elimination is an extra argument in favour of this type of experiments. Of course the interaction of benzoic acid with salicylic acid will seldom be encountered in practice, since benzoate is only used as a preservative in very small doses. Nevertheless the results obtained are important from the point of view that they reflect in general the effects of diminishing the efficiency of glycine conjugation on salicylate elimination. In our experiments half an hour before the intake of 750 mg of salicylic acid (in the form of sodium salicylate solution) 1 g of benzoate in aqueous solution was ingested followed by 250–500 mg benzoate every hour. A typical experimental result is given in figure 16.7, where in the same subject the plasma curves after sodium salicylate alone and after sodium salicylate with sodium benzoate are given. The inhibition of the elimination of salicylate by benzoate is clearly visible. Details of the dosage regimen of benzoate, together with the fitted values of the relevant pharmacokinetic parameters are given in table 16.3.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>V/F (l)</th>
<th>K_M (mg/l)</th>
<th>f</th>
<th>t_ei (hr)</th>
<th>Q_r (%)</th>
<th>pH_{ur,av} (%) dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>S</td>
<td>7.7</td>
<td>37</td>
<td>0.72</td>
<td>4.5</td>
<td>0.4</td>
<td>5.33</td>
</tr>
<tr>
<td></td>
<td>S + B</td>
<td>7.1</td>
<td>57</td>
<td>0.67</td>
<td>5.3</td>
<td>0.3</td>
<td>5.42</td>
</tr>
<tr>
<td>AL</td>
<td>S</td>
<td>7.8</td>
<td>31</td>
<td>0.84</td>
<td>3.1</td>
<td>5.6</td>
<td>5.78</td>
</tr>
<tr>
<td></td>
<td>S + B</td>
<td>7.7</td>
<td>52</td>
<td>0.84</td>
<td>4.5</td>
<td>5.4</td>
<td>5.92</td>
</tr>
<tr>
<td>HV</td>
<td>S</td>
<td>7.4</td>
<td>56</td>
<td>0.96</td>
<td>2.9</td>
<td>7.3</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td>S + B</td>
<td>7.0</td>
<td>129</td>
<td>1.00</td>
<td>4.3</td>
<td>5.7</td>
<td>6.15</td>
</tr>
</tbody>
</table>

1. S = salicylic acid, B = benzoic acid
2. \( \tau_{ei} \) refers to the ultimate straight part of the plasma curve
3. \( Q_r \) = amount of salicylic acid that is excreted in urine unchanged
4. \( pH_{ur,av} \) = weighted average of pH of all urine samples produced during each experiment

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The most striking result is the increase in $K_M$, which can be explained on basis of the fact that benzoate competes with salicylate for the mechanism that couples these drugs with glycine. A concomitantly prolonged time constant for elimination $\tau_{el}$ is observed. Further it seems to be unaffected, as is the case for the total percentage of salicylate excreted unchanged in urine. Obviously benzoate does not interfere with renal clearance. The results seem to be easily interpretable, but there is a strange aspect involved in this matter, viz. the fact that the rate of elimination of benzoate is so high that one is forced to assume that soon after benzoate administration is stopped the level of this inhibitor falls down to negligible values. As an illustration if this figure 16.8 shows the plasma curve of benzoate after oral administration of sodium benzoate in solution. In this figure also the influence of salicylate of the benzoate curve is indicated and it may be noted that benzoate elimination is hardly impaired by concurrent administration of large doses of sodium salicylate. These curves confirm the observations of Amsel and Levy (1969) that the excretion rate of

![Figure 16.8](image)

**Figure 16.8**

*Plasma curves of benzoic acid after administration of sodium benzoate alone or together with sodium salicylate. Benzoate obviously is eliminated in a non-linear way. Although salicylate has some influence on the profile of the benzoate curve, it seems that the elimination of benzoate is hardly retarded.*
hippuric acid after administration of sodium benzoate is independent of the simultaneous presence of salicylate in plasma. This means that, although salicylate is present in concentrations that are certainly higher than its Michaelis-Menten constant, the expected decrease of benzoate clearance fails to turn up. As Amsel and Levy pointed out the only logical explanation is that the formation of hippurate is limited by the rate of supply of the co-substrate glycine, which is not the case for salicyluric acid formation. However, the question remains how it is possible that the influence of benzoate administration on salicylate elimination is visible even after benzoate has disappeared from the body (which was confirmed by benzoate measurements in plasma and urine and of hippurate in urine). Although the pharmacokinetic behaviour of benzoic acid in plasma, which we are currently investigating in detail, is definitely much more complex than the simple picture that arises from urinary excretion data, we tend to ascribe the prolonged influence of benzoate to a partial exhaustion of the easily mobilized glycine pool in the body. As a matter of fact this implies that the salicylate-benzoate interaction described here is only partly competitive whereas after some time a more non-competitive interaction occurs. Preliminary calculations which we performed on such mixed interaction models indicate that they may lead to plasma curves that are formally indiscernable from a purely competitive interaction. One final point has to be mentioned. It is well-known that the renal clearance of hippuric acid involves tubular secretion. One might think of a possible inhibition of the secretion of salicylic acid by hippuric acid as a source of kinetic drug interaction. This possibility, however, can be ruled out since the excretion of hippurate after administration of this metabolite itself shows only signs of saturation at excretion rates much higher than the rates that can occur in the experiments described above. Therefore we may assume that for the hippurate formed from benzoic acid the ratio $I/K_I$ is small enough to have no or only minor consequences for the excretion of salicylic acid.

b. Probenecid
Probenecid was developed as a drug to prolong the action of penicillin by retarding the elimination of this antibiotic via competition for the tubular secretion process (Beyer et al., 1951). Also active reabsorption processes are affected by probenecid as evidenced by the fact that it is an effective uricosuric agent (Sirota et al., 1952). Probenecid appears to be a competitive inhibitor of all kinds of active transport processes and is also a potent inhibitor of some glycine conjugases (Beyer et al., 1950). In the
field of antiinflammatory drugs for instance a strong increase in the plasma level of indomethacin has been demonstrated after concomitant administration of probenecid (Skieth et al., 1968; Brooks et al., 1974). As far as salicylate is concerned, inhibition of the tubular secretion of free salicylic acid has been demonstrated (Gutman et al., 1955) and also inhibition of salicyluric acid formation may be expected to occur. When probenecid is given for uricosuric purposes the concurrent administration of salicylate is contraindicated, since salicylate causes a decrease of the uricosuric effect of probenecid. The mechanism of this interaction is not exactly known but it might be that salicylate inhibits the tubular secretion of probenecid, which actually first has to be secreted into the tubuli before it can compete with uric acid for the active reabsorption. On the other hand it seems interesting to look after the effect of probenecid on salicylate plasma levels and to determine which pharmacokinetic parameters (if any) change under the influence of probenecid. In view of the complicated patterns of elimination involved it is difficult to predict the net result of such interaction. Whereas on the one side inhibition of tubular secretion of free salicylate may be expected (as is the case with penicillin-antibiotics) on the other hand inhibition of the formation of salicyluric acid might occur. Also the formation of other metabolites might be reduced, since probenecid is known to impair the hepatic uptake of various drugs (see for instance Kenwright and Levi, 1973).

In our experiments the volunteers took single doses of 750 mg salicylic acid in the form of sodium salicylate solution. In the probenecid trials doses of 500 mg probenecid were ingested every 8 hrs over the period of 32 hrs, starting 9 hrs before salicylate intake. On the basis of the pharmacokinetic behaviour of probenecid, which is essentially non-linear (Dayton et al., 1963), plasma levels of 25 to 50 mg/l may be expected throughout the period of such a dosage schedule. The plasma curves of salicylate with and without probenecid treatment are illustrated by figure 16.9 and table 16.4 shows a compilation of the relevant data and fitted parameters. The increase in $\tau_{el}$ caused by probenecid is obvious, and it seems that this is caused by a rise of $K_M$ as well as of $f$. The amount of salicylate that is excreted unchanged seems to be diminished under the influence of probenecid, but this effect is not very strong. For instance it is remarkable that subject BC excreted more salicylate in urine during the probenecid trial than during the control experiment. The reason obviously is the fact that the average pH of his urine during the probenecid trial was a full unit higher than during the control. So it seems that urinary pH has a much more pronounced effect on salicylate excretion than probenecid adminis-
Figure 16.9
Influence of probenecid on the elimination of salicylic acid from plasma. Probenecid causes a decreased rate of elimination of salicylate. The mechanism of this interference is discussed in the text.

TABLE 16.4
Pharmacokinetics of salicylic acid after oral administration of 750 mg salicylic acid (in the form of sodium salicylate solution) with and without concurrent administration of repeated oral doses of probenecid

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>V/F</th>
<th>K_M</th>
<th>f</th>
<th>t_e</th>
<th>Q_r</th>
<th>pH_{ur,av}</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>S</td>
<td>8.6</td>
<td>33</td>
<td>0.85</td>
<td>3.1</td>
<td>1.0</td>
<td>5.37</td>
</tr>
<tr>
<td></td>
<td>S + P</td>
<td>8.1</td>
<td>65</td>
<td>0.95</td>
<td>4.6</td>
<td>0.6</td>
<td>5.74</td>
</tr>
<tr>
<td>BC</td>
<td>S</td>
<td>7.7</td>
<td>37</td>
<td>0.72</td>
<td>4.5</td>
<td>0.4</td>
<td>5.33</td>
</tr>
<tr>
<td></td>
<td>S + P</td>
<td>7.2</td>
<td>48</td>
<td>0.85</td>
<td>4.7</td>
<td>2.0</td>
<td>6.36</td>
</tr>
<tr>
<td>WR</td>
<td>S</td>
<td>8.3</td>
<td>56</td>
<td>0.78</td>
<td>2.9</td>
<td>5.3</td>
<td>5.78</td>
</tr>
<tr>
<td></td>
<td>S + P</td>
<td>7.8</td>
<td>88</td>
<td>0.90</td>
<td>4.8</td>
<td>3.1</td>
<td>6.01</td>
</tr>
</tbody>
</table>

1. S = salicylic acid, P = probenecid
2. t_e refers to the ultimate straight part of the curve
3. Q_r = amount of salicylic acid excreted in urine unchanged
4. pH_{ur,av} = weighted average of pH of all urine samples during each experiment
Nevertheless figure 16.10 provides some evidence in favour of inhibition of tubular secretion of salicylate by the presence of probenecid, but at high salicylate levels such inhibition will hardly be appreciable and probably even the opposite effect (by inhibition of active reabsorption) will prevail. In the light of these considerations it seems unlikely that the increase in $K_M$, that is observed, may be attributed to alterations in renal elimination characteristics. More likely the variation is caused primarily by interaction at the level of biotransformation. In practice probenecid-salicylate interaction will not be important for salicylate levels in single dose situations. The consequences for salicylate levels after chronic administration, however, may be significant in that a substantial increase

![Renal excretion rate versus plasma concentration of salicylic acid administered alone, or concurrently with probenecid. The data suggest an inhibition of the tubular secretion of salicylic acid by probenecid.](image)

*Figure 16.10*
of plateau levels may be brought about by probenecid. Experimental data to confirm this are not available. On the other hand it would be more profitable to have a drug at one's disposal that enhances salicylate elimination than one that impairs it, since constant and high plateau levels of salicylate can be obtained easily anyway. The following paragraph describes some preliminary trials we performed with a substance that might be expected to be of value in this respect.

c. D-glucuronolactone*
Since conjugation with glucuronic acid is a major metabolic route for salicylates one might speculate about the possibility that salicylg glucuronides, once they have been formed, run the risk of being split again by β-glucuronidase that is known to be present in liver as well as serum. When this would be the case, then the administration of an inhibitor of β-glucuronidase would lead to an enhanced efficiency of the glucuronidation and thereby to an increased rate of elimination of salicylate. Such an inhibitor might be useful for treatment of salicylate intoxications.

It is known that D-glucarolactone is a potent inhibitor of β-glucuronidase (Marsh, 1963) and several authors have recommended its use to reduce toxic effects of foreign compounds (Boyland and Williams, 1956; Brodersen and Hermann, 1953; Hartiala and Håkkinen, 1960). In mice oral glucarolactone administration has been shown to result in inhibition of β-glucuronidase in liver and kidney (Kiyomoto et al., 1963). Evidence has been reported that glucarolactone inhibits intestinal β-glucuronidase thereby reducing the extent of enterohepatic circulation of drugs that are excreted into bile in a conjugated form (Marselos et al., 1975).

In our study we used D-glucuronolactone, which is known to be rapidly absorbed and metabolized to D-glucaric acid and its lactone-form and to be an innocuous compound when ingested by man (Marsh, 1963). The levels of glucaric acid in plasma and urine were determined according to the method of Marsh (1963). In three male volunteers (age 20—22 years; body weight 71—78 kg) we determined plasma levels and urinary excretion of D-glucaric acid after ingestion of D-glucuronolactone in aqueous solution. The halflife for elimination from plasma was estimated to be 2.5—4 hrs and of the total dose administered 15—20% was recovered in

* The idea for performing these preliminary investigations originated from stimulating discussions with Dr. P.Th. Henderson and Dr. W.R.F. Notten, whose kind cooperation is gratefully acknowledged. The significance of the glucuronic acid system in drug elimination has been discussed for instance by Aarts (1968) and Notten (1975).
urine in the form of D-glucaric acid. A 2 g dose of D-glucuronolactone gives rise to peak levels of D-glucaric acid in plasma within 1 hr (15—20 mg/l, corrected for glucaric acid levels in control periods which vary from 2—5 mg/l). On the basis of these data a dosage regimen for D-glucuronolactone was adopted consisting of a first dose of 2 g, after 1.5 hrs followed by a dose of 0.5 g, which then was repeated every hour. In the two volunteers participating in the glucuronolactone-salicylate trial, this dosage schedule led to reasonably constant plasma levels of D-glucaric acid (25—35 mg/l). Salicylic acid (750 mg in the form of sodium salicylate solution) was ingested 0.5 hr after the first dose of D-glucuronolactone. Table 16.5 gives the results of the comparative pharmacokinetics of salicylate alone and in combination with D-glucuronolactone treatment. Although these data should be regarded with due reserve, since only two cases have been studied, the outcome of the trials is not encouraging in the sense that D-glucuronolactone hardly seems to influence the elimination of salicylate. The results are even contrary to what might be expected. For instance the fact that we measured an increase in the amount of salicylate which is excreted unchanged cannot be reconciled with the expectations expressed above. An attractive hypothesis would be that D-glucaric acid, like glucose and uric acid, is actively reabsorbed from urine and competes with salicylic acid for this reabsorption mechanism. However, no direct indications at all can be found for such a mechanism. When the renal excretion rate of glucaric acid is plotted against the plasma concentration

Table 16.5 gives the results of the comparative pharmacokinetics of salicylate alone and in combination with D-glucuronolactone treatment.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>V/F (l)</th>
<th>K_M (mg/l)</th>
<th>f</th>
<th>τ_el (hr)</th>
<th>Q_r (% dose)</th>
<th>pH_{ur,av}</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>S</td>
<td>8.6</td>
<td>33</td>
<td>0.85</td>
<td>3.1</td>
<td>1.0</td>
<td>5.37</td>
</tr>
<tr>
<td>HA</td>
<td>S + G</td>
<td>8.3</td>
<td>30</td>
<td>0.88</td>
<td>3.2</td>
<td>3.2</td>
<td>5.80</td>
</tr>
<tr>
<td>JM</td>
<td>S</td>
<td>8.3</td>
<td>23</td>
<td>0.81</td>
<td>2.7</td>
<td>2.1</td>
<td>6.02</td>
</tr>
<tr>
<td>JM</td>
<td>S + G</td>
<td>8.1</td>
<td>29</td>
<td>0.76</td>
<td>3.1</td>
<td>4.5</td>
<td>5.97</td>
</tr>
</tbody>
</table>

1. S = salicylic acid, G = D-glucuronolactone
2. τ_el refers to ultimate straight part of the curve
3. Q_r = amount of salicylic acid excreted in urine unchanged
4. pH_{ur,av} = weighted average of pH of all urine samples during each experiment

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(figure 16.11; derived from a typical control experiment where only glucuronolactone and no salicylate is administered), there appears to be no need at all for assuming more than purely linear excretion. In conclusion, these preliminary investigations suggest that D-glucuronolactone will not be useful for treatment of salicylate intoxications. No significant influence on plateau levels of salicylate can be expected, but if any alteration at all would occur this presumably would consist of an increase in the level. Nevertheless, in view of the literature data mentioned above, studies in man on the interaction of glucuronolactone (or glucarolactone) with other drugs that are largely glucuronidated seem to be very worthwhile.

![Renal excretion rate of D-glucaric acid as a function of its plasma concentration.](image)

*Figure 16.11*

Renal excretion rate of D-glucaric acid as a function of its plasma concentration, after oral administration of D-glucuronolactone. The excretion seems to be a purely linear process. See text for further details.
F. SOME BIOPHARMACEUTICAL ASPECTS

a. Ascal®, Rhonal®, Enterosalicyl® and sodium salicylate-sodium-bicarbonate solution.

For evaluation of analgesic therapy in both acute and chronic situations, controlled pharmacokinetic studies may provide important background information especially when different pharmaceutical formulations of the same drug have to be compared. In this connection we studied in 4 volunteers the profile of the plasma curves obtained after single dose administration of sodium salicylate in the form of solution and in the form of enteric coated tablet (Enterosalicyl®) and of acetylsalicylic acid in the form of a solution of its water soluble calcium salt (Ascal®) and in the form of coated microcrystals (Rhonal®). These formulations are extensively used in the Netherlands. Figure 16.12 gives an illustrative example of the plasma curves obtained. Figure 16.13 shows the ranges in which all individual curves for the various preparations are lying and table 16.6 contains some indices of the rate and degree of absorption of the four products. It should be noted that in case of acetylsalicylic acid containing products not the level of the drug itself but only that of its sole metabolite

![Figure 16.12](image)

*Figure 16.12*  
Comparison of plasma levels of salicylate after oral administration of two acetylsalicylic acid preparations (Rhonal® and Ascal®) and two salicylic acid preparations (Enterosalicyl® and sodium salicylate solution) to the same individual. Note that the absorption of the aqueous dosage forms is more rapid than that of the tablets.
Cf. figure 16.12. In this figure the ranges are indicated in which all concentration data of 4 subjects are situated. The differences in absorption rate seen in figure 16.12 are confirmed by these ranges.

Salicylic acid is measured. Nevertheless, this gives useful information on the absorption process, in view of the facts that acetylsalicylic acid, which is absorbed as such (see introduction), is rapidly deacetylated to salicylic acid and that all salicylic acid measured must originate from the acetylsalicylic acid administered. Therefore we may conclude that absorption from Ascal® proceeds definitely faster than from Rhonal®. Because acetylsalicylic acid is quickly deacetylated to salicylic acid, which is much less effective as an analgesic, we tend to conclude that for general analgesic purposes Ascal® should be preferred over Rhonal®. The result of this comparison is not very surprising since Leonards (1963) convincingly demonstrated that the rate of gastro-intestinal absorption of acetylsalicylic acid is markedly increased when water soluble derivatives are applied. However, also the degree of gastro-intestinal disturbances of the various acetylsalicylic acid formulations should be taken into account. Especially gastro-intestinal bleeding is a well-known risk of acetylsalicylic acid (Salter, 1968), the degree of which is strongly dependent on the pharmaceutical formulation (Wood et al., 1962; Stubbé et al., 1962; Györy and
TABLE 16.6
Time and concentration of the plasma peak of salicylic acid, obtained after oral administration of 4 different salicylate preparations

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>( t_{\text{max}} ) (hr)</th>
<th>( C_{\text{max}} ) (mg/l)</th>
<th>( t^{1/2} ) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>Sa sol</td>
<td>10</td>
<td>52.2</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>Enterosalicyl®</td>
<td>6.5</td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascal®</td>
<td>10</td>
<td>59.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhonal®</td>
<td>2.0</td>
<td>32.0</td>
<td></td>
</tr>
<tr>
<td>VF</td>
<td>SA sol</td>
<td>(&lt; 0.5)</td>
<td>51.55</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Enterosalicyl®</td>
<td>8.5</td>
<td>31.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascal®</td>
<td>2.0</td>
<td>48.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhonal®</td>
<td>3.8</td>
<td>35.5</td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>SA sol</td>
<td>(&lt; 0.5)</td>
<td>55.60</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Enterosalicyl®</td>
<td>8.5</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascal®</td>
<td>1.1</td>
<td>42.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhonal®</td>
<td>3.8</td>
<td>35.5</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>SA sol</td>
<td>(&lt; 0.5)</td>
<td>43.50</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Enterosalicyl®</td>
<td>8.1</td>
<td>29.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ascal®</td>
<td>0.9</td>
<td>40.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhonal®</td>
<td>3.8</td>
<td>38.0</td>
<td></td>
</tr>
</tbody>
</table>

1. All subjects male, age 19-24
2. SA sol = sodium salicylate solution. The salicylic acid content of the formulations is SA sol 385 mg, Ascal® 385 mg, Rhonal® 385 mg, Enterosalicyl® 431 mg.
3. \( t_{\text{max}} \) and \( C_{\text{max}} \) refer to the highest measured concentration.

Stiel, 1968, Frenkel et al., 1968, Leonards and Levy, 1967, 1969, 1972 and 1973) It appears that enteric coated formulations have the lowest risk of inducing blood loss but since these usually are quite slowly absorbed their analgesic effectiveness is questionable. Fortunately also the water soluble preparations especially when these are highly buffered are reasonably safe. Therefore these seem to be the most appropriate dosage forms to obtain acute analgesia (for instance buffered effervescent preparations). Also the kinetic comparison of the two dosage forms, containing sodium salicylate comes up to the patterns expected. The solution leads to maximal plasma levels of salicylate usually within half an hour whereas the enteric coated tablets give these maxima only after 6.5 to 8.5 hrs. These last maxima inevitably are lower than those obtained after ingestion of the solution. On the other hand it should be noticed that from 5—6 hrs after
intake the plasma level obtained from Enterosalicyl® exceeds that from the solution substantially. Here we touch an important point with regard to the evaluation of the two drug formulations. Since sodium salicylate is not suitable for general analgesic purposes, but only for treatment of inflammatory disorders, it is very questionable if high peak levels (of short duration) are of any advantage at all. More likely constant levels, lasting for long periods of time, should be preferred. Furthermore antiinflammatory therapy as a rule implies protracted administration of large amounts of drug. It is very well conceivable that in such a situation a dosage form from which the active substance is slowly released is more satisfactory than a solution. As far as the gastro-intestinal side-effects are concerned, the pharmaceutical formulation is less critical in case of sodium salicylate than in case of acetylsalicylic acid. Gastro-intestinal blood loss after sodium salicylate is much less significant than after acetylsalicylic acid and is often not observed at all (see references cited above). Especially enteric coated tablets can be regarded as safe in this respect. Blood levels on chronic administration will be discussed in the following part of this chapter (16 G). Our data do not allow definite statements on the biological availability of the four products under investigation. Nevertheless the biological availability of the aqueous solutions (Ascal® and sodium salicylate solution) must be practically complete in view of the height of the peak levels obtained and the apparent volume of distribution that may be derived (4.5–6.5 l). Complete availability of acetylsalicylic acid from aqueous solution has been confirmed by Rowland et al. (1972), who also showed that this does not imply that the total dose of acetylsalicylic acid reaches the general circulation intact (Rowland estimated the fraction that was deacetylated before passage into the general circulation at 30%). Under the assumption of constant pharmacokinetic behaviour within the same individual over the whole period of experimentation (1 month), in our study the average bioavailability of Rhonal® and Enterosalicyl® may be estimated at 85% of that of the corresponding water soluble formulations. The difference, however, is not statistically significant. Finally, it should be mentioned that the general characteristics, that we considered here as determining the absorption process of salicylates, appear to be valid in many similar comparative studies (see for instance Bell et al., 1966; Pütter and Bauer, 1970; Graham and Rowland, 1972; Hollister, 1972; Frislid et al., 1972).

b. Salipyrin
Salipyrin (phenazone salicylate) is a complex molecular compound
of phenazone and salicylic acid. It is not a salt, the carboxylic function of salicylic acid is not neutralized (Huckel, 1962). In the Netherlands salipyrin is quite extensively used as a weak antipyretic analgesic for the relief of less severe types of pain and fever. Although salipyrin should be regarded as an administration form of phenazone rather than of salicylic acid, we report the results of our investigations into the kinetic behaviour of this drug in this chapter since we were primarily interested in the factors that modify the absorption characteristics of salicylate. (It must be stressed that in view of the large difference in the rate of elimination between salicylate and phenazone, salipyrin can never be a suitable dosage form for chronic administration of salicylate). This study consisted of the administration of three different preparations to four volunteers: 1 g salipyrin in aqueous suspension and equimolar doses of sodium salicylate and phenazone in aqueous solution. Figure 16.14 shows an illustrative example of the plasma curves obtained in the same individual after each of the three formulations. Table 16.7 gives some relevant pharmacokinetic data for all volunteers. Our data reveal no difference between biological

![Graph](image)

**Figure 16.14**

**Plasma curves of salicylic acid and phenazone after administration of 1 g salipyrin as compared with plasma curves after each drug separately in equimolar doses. It may be noted that the curves for salicylic acid are practically the same, whereas for phenazone the plasma levels differ markedly. This phenomenon is discussed in detail in the text.**

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**TABLE 167**

Pharmacokinetic parameters obtained for salicylic acid and phenazone after oral administration of 1 g salipryn (SAP) as aqueous suspension and of equimolar amounts of sodium salicylate (SA) or phenazone (P) in aqueous solution

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>Salicylic acid</th>
<th>Phenazone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>V/F (1) k&lt;sub&gt;Ce&lt;/sub&gt;/F (ml/min) τ&lt;sub&gt;el&lt;/sub&gt; (hr) t&lt;sub&gt;1/2&lt;/sub&gt; (hr) t&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>V/F (1) k&lt;sub&gt;Ce&lt;/sub&gt;/F (ml/min) τ&lt;sub&gt;el&lt;/sub&gt; (hr) t&lt;sub&gt;1/2&lt;/sub&gt; (hr) t&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
</tr>
<tr>
<td>GB</td>
<td>SAP</td>
<td>7 6 42 30 21 1 50 67 84 0 75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>7 5 41 30 21 0 75 45 32 1 00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>7 5 36 5 24 1 25 70 43 1 00</td>
<td></td>
</tr>
<tr>
<td>WD</td>
<td>SAP</td>
<td>7 5 36 3 5 24 1 25 50 31 1 00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>7 7 37 3 5 24 &lt; 0 25 70 43 1 00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>7 7 37 3 5 24 &lt; 0 25 50 31 1 00</td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>SAP</td>
<td>6 9 36 3 2 22 1 25 47 50 1 00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>6 9 36 3 2 22 &lt; 0 25 47 50 1 00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>6 9 36 3 2 22 &lt; 0 25 47 50 1 00</td>
<td></td>
</tr>
<tr>
<td>JS</td>
<td>SAP</td>
<td>7 0 33 3 6 25 1 75 56 49 1 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>7 0 33 3 6 25 0 75 47 42 1 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>7 0 33 3 6 25 0 75 47 42 1 50</td>
<td></td>
</tr>
</tbody>
</table>

1. All subjects male, age 20-23 yr, body weight 69-77 kg
2. Parameters calculated on basis of free salicylic acid, assuming linear pharmacokinetics

Availability of salicylic acid from aqueous sodium salicylate solution and that from salipryn. The only difference appears to be that maximal plasma levels are more rapidly reached after ingestion of the sodium salicylate solution and that these accordingly are somewhat higher than after salipryn. A different result arises with regard to phenazone. Here it is not so much a time shift in the peak concentration, but a difference in biological availability that is predominant. In three of the four subjects the biological availability of phenazone from salipryn was lower than from aqueous phenazone solution, the ratios being 0.85, 0.67 and 0.71. In the fourth person no difference was observed. It may be noted that these data strongly support the idea that salipryn is split into its components already in the gastro-intestinal tract. The pattern of elimination is remarkably constant in all cases, except the elimination of phenazone in subject B. The enormous variation in the rate of elimination of phenazone in this subject (in two weeks time) is totally inexplicable and contrary to the
common experience of a very constant kinetic behaviour of phenazone (see chapter 10). No attempts were made to characterize the metabolic pattern of the subject under consideration in more detail. From a pharmacokinetic point of view, the data presented do not provide a rational basis for the use of salipyrin as a dosage form of phenazone (or salicylate). As a matter of fact a discouragement of its use would be more justified. On the other hand it cannot be excluded that the specific combination as present in salipyrin may have beneficial effects in some cases or that it may be better tolerated than its components separately by some patients.

G. REPEATED ADMINISTRATION

For the treatment of various rheumatic diseases plasma levels of salicylate as high as 300 mg/l are required. These levels are only slightly below the level at which centrally mediated side-effects become predominant (see for instance Goodman and Gillman, 1970). Since the plasma concentration has to be maintained at this slightly subtoxic level for long periods of time, the pharmacokinetic behaviour of salicylate on chronic administration deserves special attention. In chapter 15 the pharmacokinetic theory of the consequences of capacity-limited elimination for chronic drug administration has been outlined. Paulus et al. (1971) reported a three-fold increase in plasma salicylate level after increasing the daily dose of acetylsalicylic acid from 65 to 100 mg per kg body weight. Levy and Tsuchiya (1972) presented some numerical calculations in order to account for this phenomenon. Obviously a qualitative explanation is very simple on basis of the capacity-limited features of salicylate elimination. It is likely, however, that generalized quantitative predictions will be impossible in view of the large interindividual variation in the kinetics of salicylate metabolism (Gibson et al., 1975; Gupta et al., 1975). In this paragraph we report experimental data to illustrate the theory and we will discuss these data with special reference to the simple infusion-approach that we proposed in chapter 15.

a. Relationship between dose and plateau level of salicylate

Figure 16.15 shows the profile of the plasma concentration curve over a 8 hr dosage interval at a dose of 1 g sodium salicylate (in solution) in a steady state situation and the change that is brought about by diminishing the dose per interval to 0.5 g. When the average plateau concentration over the dosage interval is estimated, these turn out to 142–29 and 109–27 for the subjects K and Z respectively. So in these cases doubling of the dose
Figure 16.15
Plasma levels of salicylate over a dosage interval (8 hrs) in plateau situations after a daily dose of 3 g (left) and the change that is brought about by halving the dose (right). The difference in the average plateau concentration is as much as a factor 4–5, whereas in linear pharmacokinetics a factor 2 would be expected.
results in a 4- to 5-fold increase in average steady state level. On basis of equation 15.10 it is possible to estimate the values of both $K_M$ and $f$ when the average plateau levels at two different dosage regimens are measured, provided that the maximally attainable total body clearance constant $k_{Cel}$ is known. From the profile of the plasma curve during the 0.5 g dosage regimen an estimate of $k_{Cel}$ can be made, for the subjects K and Z, although the estimates probably will be too low in view of the clear signs of non-linear behaviour of the plasma concentration. Assuming that $k_{Cel}$ is in the order of 3 l/hr, $K_M$ may be estimated at about 50 mg/l for both subjects K and Z with $f$ being near to unity. These values seem to be in a quite normal range. However, the estimation is very much dependent on the average plasma level at the low dosage regimen and it can easily be derived that small variations in this average level will profoundly influence the calculated $K_M$ and $f$ values. More reliable estimates can be made when equation 15.10 is applied to experimental data in which higher doses have been administered. Table 16.8 shows the average plateau concentrations reached in a volunteer who took 0.75 g salicylic acid (as sodium salicylate solution) three times daily for a week, followed by three times daily 1 g for another week. After the last 1 g dose the decay of the plasma curve was followed for 30 hrs in order to be able to calculate the metabolic and kinetic parameters. These parameters are also given in table 16.8, together with the $K_M$ and $f$ value estimated from the average plateau levels according to equation 15.10 and using $k_{Cel} = 2.4$ l/hr. The values for $K_M$ and $f$ that are calculated by these two procedures agree reasonably well. It should be noticed, however, that especially the magnitude of $f$ is very critical with regard to the accumulation characteristics (see also chapter 15). This implies that only when $f$ could be estimated with high precision, predictions may be made concerning the height of the plateau

<table>
<thead>
<tr>
<th>$D/\Delta t$ (mg/hr)</th>
<th>$\bar{C}_{pl}$ (mg/l)</th>
<th>$K_M$ (mg/l) and $f$ estimated on basis of $\bar{C}_{pl}$</th>
<th>$K_M$ (mg/l) and $f$ obtained after last dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>750/8</td>
<td>98</td>
<td>$K_M = 25$</td>
<td>$K_M = 38 \pm 60%$</td>
</tr>
<tr>
<td>1000/8</td>
<td>149</td>
<td>$f = 0.76$</td>
<td>$f = 0.80 \pm 20%$</td>
</tr>
</tbody>
</table>

TABLE 16.8
Average plateau concentration ($\bar{C}_{pl}$) in a subject (JBi) who took 750 mg salicylic acid three times daily for a week and then a dose of 1000 mg for another week. After the last dose the plasma curve was followed and the parameters were calculated. See text for further explanation.
level. Unfortunately \( f \) is not a constant within one individual. Already in part D of this chapter it has been shown that \( f \) is very sensitive to changes in urinary pH. As a consequence the plateau levels of salicylate during chronic therapy are highly dependent on the pH of the urine. An increase in this pH will lead inevitably to a decrease in \( f \) and decrease in steady state plasma level. This phenomenon has been observed by Levy and Leonards (1971) and Levy et al., (1975), and is very important for chronic therapy. On the one side a slight decrease in the pH of the urine may cause toxic plasma levels in a patient on a dosage regimen which first yielded therapeutic and subtoxic levels. On the other hand an increase in urinary pH may change a dosage regimen from adequately therapeutic into insufficient. For instance the concomitant use of antacids may have profound influence (Gibaldi et al., 1974; Levy et al., 1975). Further alkalinization of urine is an important therapeutic measure for the treatment of salicylate intoxication (see for instance Morgan and Polak, 1971; Hill, 1973; Bender, 1975). Infusion of sodium bicarbonate may enhance the elimination of salicylate very effectively, especially in case of very high plasma levels.

The importance of fluctuations in urine pH, even in relatively short-term experiments may be illustrated by figure 16.16 where the plasma curve is depicted as it was obtained in a subject (JBo) after administration of 1 g salicylic acid (as sodium salicylate solution) three times daily for 9 days. After the last dose again the plasma curve was followed for 24 hrs. The average plateau level was about 160 mg/l. In the right part of the figure the drawn line represents the result of the curve fitting procedure and the parameters are given. When on basis of the fitted parameters the theoretical mean plateau concentration is calculated (top of right axis) it appears that the measured plateau concentration corresponds to an \( f \) value of about 0.85, whereas the curve on day 10 revealed an \( f \) value of 0.97. This appears to be the consequence of differences in urinary pH on day 10 when no further sodium bicarbonate was ingested, and the days before, when sodium bicarbonate was present in the solution that was taken.
Figure 16.16
Repeated administration of 1 g salicylic acid three times daily. The line in the left part connects plasma concentrations measured just before intake of the following dose. Theoretical plateau concentrations for various $f$ values are given (top of right axis). The average plateau level measured corresponds with an $f$ value of about 0.85, whereas the curve after the last dose reveals an $f$ value of 0.97. This difference is probably related to variations in the pH of the urine, as discussed in the text.
Biopharmaceutical aspects

Figure 16.16 points at the significance of the dosage form for the accumulation characteristics of salicylate. This significance is substantiated by fig 16.17 and table 16.9. Figure 16.17 illustrates the difference in steady state levels measured in a female patient who was taking 1.5 g sodium salicylate (1.3 g salicylic acid) 4 times daily, first in the form of Enterosalicyl® tablets and after some weeks in the form of a bicarbonate containing aqueous solution. Whereas in case of Enterosalicyl® administration very constant plateau levels of about 390 mg/l are reached, the levels after salicylate solution are much more fluctuating and much lower (averaging around 220 mg/l). The fluctuating levels in the last case may be explained partly by a more rapid absorption of salicylate from the solution but will be associated with the non-linear kinetic behaviour of salicylate as well. Obviously the capacity-limitation implies a decrease in the rate of elimination with increasing plasma levels, so that the plasma curve over each interval will exhibit less fluctuation, as the level rises.

![Graph showing plasma levels of salicylic acid](image)

**Figure 16.17**

Plasma levels of salicylic acid in a female subject after chronic administration of 1.5 g sodium salicylate 4 dd in the form of enteric coated tablets (left) and aqueous solution (right). Different symbols refer to different measuring periods for each form. Obviously the plateau is reached. It is remarkable that the same dose of sodium salicylate gives much lower plasma levels when administered as a solution than when administered as enteric coated tablets. See text for details.
Biopharmaceutical aspects of chronic salicylate administration. The drugs were administered in equal dosages per interval

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dosage from</th>
<th>Daily dose $^1$ (g)</th>
<th>$\Delta t^2$ (hr)</th>
<th>$\bar{C}_{pl}^3$ (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>Enterosalicyl®</td>
<td>5.2</td>
<td>6</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>solution</td>
<td>5.2</td>
<td>6</td>
<td>390</td>
</tr>
<tr>
<td>J</td>
<td>Enterosalicyl®</td>
<td>2.6</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>solution</td>
<td>2.6</td>
<td>8</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>Rhonal®</td>
<td>2.3</td>
<td>8</td>
<td>130</td>
</tr>
<tr>
<td>G</td>
<td>solution</td>
<td>2.6</td>
<td>8</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Rhonal®</td>
<td>2.3</td>
<td>8</td>
<td>95</td>
</tr>
</tbody>
</table>

1. dose expressed in g free salicylic acid  
2. $\Delta t =$ dosage interval  
3. $\bar{C}_{pl} =$ average plateau concentration of salicylic acid in plasma

Table 16.9 summarizes some data on the magnitude of the accumulation plateau that is obtained on chronic administration of various dosage forms of salicylate. As far as salicylate levels are concerned Rhonal® and Enterosalicyl® seem to be equivalent at least in the few measurements we did. Although the average plateau level usually is lower for Rhonal® than for Enterosalicyl® the difference can be fully accounted for by the fact that Rhonal® contains a lower dose when expressed as salicylic acid (385 mg and 431 mg for Rhonal® and Enterosalicyl® respectively). In any case the solution is clearly inferior. In view of the non-linear pharmacokinetics of salicylate the difference will become more pronounced with increasing daily dose. The relatively low salicylate levels that are encountered with the solution most probably are caused by the addition of sodiumbicarbonate to the solution as argued before.

The data presented in this paragraph stress the importance of pharmaceutical formulation and possible comedication for the evaluation of dosage regimens of salicylate, needed for adequate antiinflammatory therapy. Our analysis indicates that there are some possibilities for predicting levels after chronic medication but it also points at some weak aspects of the theoretical approach, with special reference to the uncertainty of urinary pH. Although explanation of the findings afterwards is reasonably successful the predictive value is limited in view of inter- and intra-individual variables that cannot easily be incorporated into a general theoretical approach.
REFERENCES


Pharmacokinetics is the study of the behaviour of drugs in man or animals. It includes the kinetic processes of diffusion in and out various compartments, with elimination usually assumed to occur from a central compartment. In general, all kinetic processes are assumed to be first order, so that the rate of drug transfer is supposed to be directly proportional to the drug concentration in the compartments. The kinetic processes may then adequately be described by a set of linear differential equations (linear pharmacokinetics, section II).

In drug elimination, however, there are several processes involved, which are in principle saturable (e.g. metabolic transformation and renal tubular secretion). Such processes can be expected to lead to deviations from first-order pharmacokinetics. Non-linear pharmacokinetics which is the topic of section III deals with all processes that cause deviations from first-order behaviour. There are, however, only a few mechanisms for which a better mathematical description than the first-order approximation is possible.

In chapter 13 the pharmacokinetic behaviour of foreign substances that are completely or partially eliminated via metabolism by saturable enzyme systems is analysed. General integrated equations are derived which describe the time course of the plasma concentration under the assumption of a saturable enzyme system according to Michaelis-Menten kinetics in combination with normal first-order elimination processes. A procedure for the estimation of initial values of the elementary kinetic parameters on the basis of the models is outlined. These initial values have been used in a non-linear curve-fitting program in order to obtain reliable kinetic and enzyme parameters from the plasma curves. With these methods, kinetic and apparent enzyme parameters are calculated for ethanol, salicylic acid, 4-hydroxybutyric acid and phenytoin. The same methods were applied for the analysis of 4-aminophenazone data in chapter 10 (section II). In chapter 13 also the analogy between the mathematical description of saturable metabolism and that of tubular secretion is discussed. Further it is indicated that the influence of protein binding on the apparent total body clearance is opposite to what is found in case of Michaelis-Menten kinetics, so that in some cases capacity-limited pharmacokinetics might be concealed by protein binding.
In *chapter 14* the mathematics of the simultaneous occurrence of two capacity-limited pathways is outlined. Since for several drugs (salicylic acid, ethanol) it is known that at least two potentially saturated metabolic pathways are involved such a theoretical discussion is relevant in order to find out if, despite of the two combined mechanisms, the mathematical description of chapter 13 may be applied. The answer is positive as far as salicylic acid is concerned. It is argued that in most cases it is impossible to discriminate the two pathways on basis of plasma curves alone and that the pooling of the two routes leads to reasonably constant operational enzyme parameters, certainly for salicylic acid.

*Chapter 15* deals with the consequences of capacity-limited elimination for drug accumulation. Obviously the fact that the rate of elimination of drug decreases at increasing plasma concentrations causes a disproportionately high accumulation of the drug with increasing dose. This phenomenon is very important in practice for instance for phenytoin and salicylates. Simple formulas are presented for calculating the average plateau level that can be expected when these drugs are administered in a fixed dosage regimen for long periods of time.

*Chapter 16* is devoted to several aspects of the pharmacokinetics and biopharmaceutics of salicylates in man. The profile of the plasma curve of salicylic acid after single doses is concentration-dependent and can be characterized according to the methods of chapter 13. The volume of distribution is in the same order of magnitude as found for ibuprofen and alclofenac; also the clearance at low doses is comparable to that of these drugs. At higher doses, however, the clearance decreases by partial saturation of the enzyme systems responsible for elimination of salicylic acid. The factors involved in these processes are discussed in detail. Evidence is presented to support the view that also the renal clearance of salicylic acid is essentially non-linear. As a matter of fact indications are found for the occurrence of tubular secretion as well as active tubular reabsorption.

This complex renal clearance provides insight in the mechanism of the so-called paradox effect of salicylates on uric acid excretion. The excretion of unchanged salicylic acid in urine is highly pH dependent. Alkalinization of the urine (by administration of sodium bicarbonate) causes a higher renal clearance of unchanged salicylic acid and thereby less metabolic transformation. This implies that the influence of capacity-limited elimination becomes less pronounced. Acidification of the urine (by administering ammonium chloride) has the opposite effect. Some paragraphs of *chapter 16* deal with pharmacokinetic interactions of salicylic acid with substances suspected to alter salicylate disposition: benzoic acid,
probenecid and D-glucuronolactone. Benzoic acid appears to inhibit the metabolism of salicylic acid both competitively and non-competitively. Probenecid has an influence on the renal excretion of salicylic acid as well as on the metabolism. D-glucuronolactone appears to enhance the renal excretion of unchanged salicylic acid, whereas we expected an increase of the amount of salicylic acid excreted as glucuronide.

Further experiments are described on the non-linear kinetics after chronic administration of salicylates. It was found for instance that doubling of the daily dose of sodium salicylate from 1.5 to 3 g led to a four- to five-fold increase in the average plasma plateau concentration. Obviously such an increase cannot be explained on the basis of linear kinetics, but it fits very well within the model of capacity-limited elimination.

Also some biopharmaceutical aspects were studied. Plasma curves were compared after single administration of 500 mg sodium salicylate as a solution (containing sodium bicarbonate) and as enteric coated tablet (Enterosalicyl®). The absorption from the solution was much faster than from the tablets and the biological availability tended to be somewhat higher. However, after chronic ingestion of equal doses plateau concentrations were much higher for the tablet form than for the solution. Most likely this phenomenon has to be explained by the fact that in the solution (and not in the tablets) sodium bicarbonate is present, which increases the renal excretion of unchanged salicylic acid, thereby reducing the fraction that is metabolized by capacity-limited systems. In chronic dosage regimens Rhonal®, which is a formulation of acetylsalicylic acid (coated microcrystals), has the same characteristics as the enteric coated tablets, at least when the difference in salicylic acid content is taken into account.

In single dose situations two acetylsalicylic acid formulations were compared: Ascal®, the water soluble calcium acetylsalicylate, and Rhonal®. As expected, the water soluble form was more rapidly absorbed and it is stated that for acute analgesia only water soluble acetylsalicylic acid preparations should be used since only these may provide effective plasma levels of the analgetically superior acetylsalicylic acid, whereas less rapidly absorbed dosage forms are only salicylic acid formulations with a higher risk of side-effects than sodium salicylate itself. Finally the drug salipyrin, a molecular compound of salicylic acid and phenazone, has been compared with its two constituting drugs. Evidence is presented that the drug is split into its components already in the gastro-intestinal tract so that phenazone and salicylate are separately absorbed. From a pharmacokinetic point of view the use of salipyrin is not sensible, neither as a dosage form of salicylic acid, nor as a phenazone formulation.
SAMENVATTING

Dit proefschrift is gewijd aan de farmacokinetiek van een aantal antipyretische en antiflogistische analgetica bij de mens. De verschillende geneesmiddelen worden besproken vanuit een fundamenteel farmacokinetisch oogpunt, maar de praktische consequenties van de experimentele resultaten worden zoveel mogelijk aangegeven. Het geheel is verdeeld in 3 secties:

Sectie I (hoofdstukken 1-3) omvat een algemene inleiding en de beschrijving van de voornaamste methodieken die bij de onderzoeken in gebruik.

Hoofdstuk I bestaat uit een kort overzicht van de hypothesen betreffende de werking van antipyretische en antiflogistische analgetica. Tevens wordt de plaats van farmacokinetische processen in het geheel van de werking van geneesmiddelen besproken. Verder wordt het belang van farmacokinetische gegevens voor optimalisering van de farmacotherapie aangeduid, waarbij speciaal aandacht wordt besteed aan de onderzoeken die in het proefschrift worden beschreven.

In hoofdstuk 2 wordt een overzicht gegeven van de toegepaste methoden voor extractie en kwantitatieve analyse van de verschillende geneesmiddelen in biologische monsters. De meeste stoffen zijn gaschromatografisch bepaald, eventueel na derivatiseren.

Salicylzuur en flufenaminezuur werden fluorometrisch bepaald. Ook de mathematische analyse van plasmacurves volgens bepaalde farmacokinetische modellen wordt in dit hoofdstuk besproken. In het algemeen is deze analyse uitgevoerd via computerfit m.b.v. het niet-lineaire regressie programma Farmfit.

Hoofdstuk 3 geeft enige fysisch-chemische eigenschappen (verdelingscoëfficiënt en pK_a) voor de onderzochte verbindingen. Ook wordt het belang van deze parameters voor het farmacokinetisch gedrag bediscussieerd. Met nadruk wordt gewezen op de noodzaak om bij deze overwegingen de invloed van ionisatie en lipofiliteit in samenhang te beschouwen.

Sectie II (hoofdstukken 4-12) is gewijd aan de processen van absorptie en eliminatie van een aantal analgetica, voor zover deze voldoen aan lineaire kinetiek. De eliminatiesnelheid van geneesmiddelen uit het lichaam wordt hoofdzakelijk bepaald door twee farmacokinetische parameters: de
klaring, die een maat is voor de efficiency waarmee lever en nieren (en eventueel andere organen) een bepaalde stof uit het lichaam verwijderen, en het verdelingsvolume dat bepaalt hoe groot het (schijnbare) volume is waaruit de stof geëlimineerd moet worden. De gebruikelijke benadering is om alle kinetische processen als eerste orde te beschouwen, zodat de snelheid waarmee een geneesmiddel uit een bepaald compartiment verdwijnt recht evenredig is met de concentratie in dat compartiment. In dit geval kunnen alle snelheidsprocessen eenvoudig beschreven worden met behulp van een stelsel van lineaire differentiaalvergelijkingen en spreekt men van lineaire farmacokinetiek. Voor de meeste geneesmiddelen blijkt deze benadering in eerste instantie toereikend.

In hoofdstuk 4 worden de basale begrippen en de algemene mathematische formuleringen van lineaire farmacokinetische modellen in detail uitgezet. Ook wordt aandacht besteed aan de mogelijkheid van cumulatie van geneesmiddelen in het lichaam, wanneer deze chronisch toegediend worden volgens bepaalde doseringsschema's. De overige hoofdstukken in sectie II zijn gewijd aan de specifieke analgetica afzonderlijk. Ibuprofen (Brufen®) en alclofenac (Mirvan®) zijn het onderwerp van hoofdstuk 5 en hoofdstuk 8 respectievelijk. Deze beide middelen hebben een klein verdelingsvolume (<10 l), kleiner nog dan het volume van de extracellulaire vloeistof, hetgeen waarschijnlijk veroorzaakt wordt door binding aan plasma-eiwitten. Ook de klaringsconstante van deze stoffen is klein (30-50 ml/min), maar vanwege het kleine verdelingsvolume is hun halfwaardetijd toch nog vrij kort (1.5-3 uur). Hoewel de totale klaring van ibuprofen en alclofenac ongeveer gelijk is, bestaan er grote verschillen in de relatieve bijdragen van renale en metabole klaring. Ibuprofen wordt nauwelijks onveranderd uitgescheiden in de urine, terwijl van alclofenac soms hoeveelheden tot 50% van de dosis onveranderd in de urine verschijnen. Deze verschillen hangen samen met verschillen in fysisch-chemische eigenschappen (wateroplosbaarheid, vetoplosbaarheid, eiwitbinding). De renale klaring van alclofenac neemt sterk toe met toenemende urine-productie, maar lijkt overigens te bestaan uit uitsluitend filtratie en passieve terugresorptie. Voor ibuprofen worden naast deze lineaire processen, duidelijke aanwijzingen voor een tubulair secretiemechanisme aangetroffen, hoewel dit mechanisme slechts een geringe capaciteit heeft en snel verzadigd is. Beide geneesmiddelen worden na orale toediening vrij snel geresorbeerd. De beide handelsvormen van alclofenac (Mirvan® tabletten en suppositoria) zijn praktisch equivalent in dit opzicht. Zoals verwacht op grond van de farmacokinetische parameters, blijkt noch ibuprofen, noch alclofenac aanleiding te geven tot cumulatie bij toediening driemaal daags.
In hoofdstuk 6 wordt de farmacokinetiek van de afzonderlijke optische isomeren van ibuprofen besproken. De resultaten ondersteunen het idee dat in het menselijk lichaam inversie van levo- naar dextro-ibuprofen voorkomt, terwijl de omgekeerde reactie niet of nauwelijks plaatsvindt.

Hoofdstuk 7 beschrijft een vergelijking van de farmacokinetiek van ibuprofen en ibufenac. Het verdelingsvolume van ibufenac lijkt wat groter te zijn dan dat van ibuprofen. Ibufenac wordt veel langzamer geresorbeerd en doorgaans ook minder snel geëlimineerd dan ibuprofen. Er werden aanwijzingen gevonden voor een enterohepatische circulatie van ibufenac.

Het onderwerp van hoofdstuk 9 is het farmacokinetisch gedrag van flufenaminezuur (Arlef®) en mefenaminezuur (Ponstan®). Deze beide fenamaten vertonen tamelijk onregelmatige plasmacurves in de zin dat het onmogelijk bleek de curves redelijk te beschrijven volgens normale modellen. Relatieve waarden voor de klaringsconstanten werden verkregen m.b.v. het oppervlak onder de plasmacurve. De twee fenamaten zijn opvallend weinig wateroplosbaar. Vooral voor mefenaminezuur lijkt dit moeilijkheden in het resorptieproces te veroorzaken. De resorptie van deze stof wordt bevorderd door voedsel. Een emulgerend effect van de geproduceerde gal zal hieraan wellicht ten grondslag liggen.

Hoofdstuk 10 is gewijd aan een aantal fenazon-derivaten. Typisch voor het kinetisch gedrag van fenazon en zijn derivaten is de grote inter-individuele variatie. Zelfs in de homogene groep van jonge vrijwilligers die wij bestudeerden bleek een veel grotere farmacokinetische variatie voor fenazon-derivaten dan voor de aromatische zuren. Het verdelingsvolume van fenazon-derivaten is ongeveer gelijk aan het volume van het totale lichaamswater (30-40 l), maar lijkt iets groter te zijn voor meer lipofiele derivaten, zoals 4-isopropylfenazon (propyfenazon). Er komen in deze serie grote verschillen in klaringsconstante voor: van 30-50 ml/min voor fenazon tot 500 ml/min of meer voor 4-isopropylfenazon of 4-amino-fenazon (4-aminoantipyrine). De renale klarining is meestal verwaarloosbaar (0-6% onveranderd uitgescheiden in de urine).

Invoering van een isopropyl-groep in fenazon leidt tot een veel snellere metabole eliminatie (waarschijnlijk via oxidatie van de isopropyl-groep). Dit geldt ook voor substitutie van een dimethylamino-groep, waardoor 4-dimethylaminofenazon (amidopyrine, Pyramidon®) ontstaat. Ook hier is de grotere klarining het gevolg van metabole instabiliteit van de substituent, omdat het metabolisme vooral via N-demethylering plaatsvindt. De eerste gedemethyleerde metaboliet, 4-methylaminofenazon, wordt minder snel geëlimineerd dan het dimethylderivaat zelf. Het feit dat 4-isopropylaminofenazon (Isopyrin®) weer sneller geëlimineerd wordt dan 4-dimethyl-
aminofenazon wijst erop dat de isopropyl-groep sneller verwijderd kan worden dan een methyl-groep. De klaring van 4-aminofenazon is niet-lineair. Hoewel de niet-lineaire farmacokinetiek het onderwerp is van sectie III, is deze stof vanwege zijn chemische structuur toch in hoofdstuk 10 opgenomen. 4-aminofenazon wordt voor een aanzienlijk deel (soms tot 40%) geëlimineerd via renale excretie, hetgeen ongebruikelijk is voor fenazon-derivaten, en verder via N-acetylering. De bijdrage van de renale en metabole processen aan de totale (niet-lineaire) klaring wordt in detail besproken. Speciale aandacht wordt besteed aan de mogelijkheid dat ook voor deze stof een genetisch bepaald verschil tussen snelle en langzame acetyleerders optreedt.

In hoofdstuk 11 worden in het kort een aantal farmacokinetische gegevens voor fenacetine en paracetamol besproken. In gebruikelijke dosis (≤ 500 mg) leidt fenacetine tot nauwelijks meetbare, zeer lage plasmaspiegels. Dit verschijnsel wordt niet veroorzaakt door een groot verdelingsvolume, maar door een sterk first-pass effect. Paracetamol vertoont dit verschijnsel niet. Mede op basis van biofarmaceutische overwegingen lijkt paracetamol voor de pijntherapie waardevoller dan fenacetine.

Sectie III is geheel gewijd aan de theoretische en praktische aspecten van de niet-lineaire farmacokinetiek. Bij de eliminatie van geneesmiddelen zijn duidelijk processen betrokken, die in principe verzadigbaar zijn (enzymatische omzetting en tubulaire secretie bijv.). Onder bepaalde omstandigheden zullen daardoor afwijkingen van eerste-orde gedrag optreden.

In hoofdstuk 13 wordt de kinetiek van stoffen, die geheel of gedeeltelijk via metabolisme door verzadigbare enzymsystemen (capaciteitsbeperkte kinetiek) worden geëlimineerd, geanalyseerd. Er wordt een grafische procedure beschreven om te komen tot een schatting van de elementaire kinetische parameters op basis van modellen met geheel of gedeeltelijk capaciteitsbeperkte eliminatie. De aldus gevonden waarden worden gebruikt als beginschatting voor computerfit, zoals gedemonstreerd aan de hand van een aantal voorbeelden (ethanol, salicylzuur, 4-hydroxyboterzuur en phenytoine). Dezelfde methode werd toegepast op 4-aminofenazon (hoofdstuk 10).

In hoofdstuk 13 wordt ook gewezen op de volkomen analogie tussen tubulaire secretie en verzadigbaar metabolisme. Verder wordt aangegeven dat eiwitbinding een effect heeft op de klaring dat tegengesteld is aan het effect van capaciteitsbeperkte kinetiek.

In hoofdstuk 14 wordt de theorie van twee gelijktijdig voorkomende capaciteitsbeperkte wegen behandeld. Er wordt aangetoond dat in veel gevallen (zeker ook voor salicylzuur) de analyse volgens hoofdstuk 13 ge-
oorloofd is, ondanks het feit dat meerdere verzadigbare mechanismen voor eliminatie parallel functioneren. Verder wordt besproken dat het in de meeste gevallen onmogelijk zal zijn de verschillende mechanismen experimenteel te onderscheiden op basis van plasmametingen alleen.

Hoofdstuk 15 behandelt de gevolgen van capaciteitsbeperkte eliminatie voor cumulatie van geneesmiddelen. Het is duidelijk dat deze capaciteitsbeperking, die ertoe leidt dat de eliminatie van de stof met toenemende concentratie afneemt, een onevenredig sterke verhoging van de plateauconcentratie met toenemende dosis veroorzaakt. Speciaal voor salicylaten, ethanol en phenytoine is dit verschijnsel van uitermate groot belang. Eenvoudige formules worden gegeven, die het mogelijk maken om gemiddelde plateauconcentraties te berekenen, zonder toepassing van numerieke methoden.

Hoofdstuk 16 beschrijft verschillende aspecten van de farmacokinetiek en biofarmacie van salicylaten bij de mens. Het profiel van de plasmacurve van salicylzuur na enkelvoudige dosering is concentratie-afhankelijk en kan beschreven worden m.b.v. de methoden die zijn aangegeven in hoofdstuk 13. Het verdelingsvolume is vergelijkbaar met dat van ibuprofen en alclofenac en ook de klaring bij lage doseringen is in dezelfde orde van grootte als die van deze geneesmiddelen. Bij hogere doseringen neemt de klaring echter af als gevolg van gedeeltelijke verzadiging van de eliminerende enzymen. De factoren, die bepalend zijn voor deze verschijnselen, worden in detail besproken. Onder bepaalde omstandigheden blijkt ook de renale excretie van salicylzuur duidelijk niet-lineair te zijn. Bij lage urine-pH worden aanwijzingen gevonden voor zowel tubulaire secretie als actieve terugresorptie. Een dergelijk complex patroon is in overeenstemming met het paradox effect van salicylzuur op de uraat-excretie. De excretie van onveranderd salicylzuur neemt sterk toe met toenemende urine-pH (verminderd van de passieve terugresorptie) zodat bij hoge pH de actieve processen overschaduwd worden door de passieve, lineaire processen. Wanneer door toediening van natriumbicarbonaat of ammoniumchloride de urine resp. basisch of zuur gemaakt wordt, blijkt het verschil tussen beide behandelingen vooral te bestaan uit een verandering van de hoeveelheid onveranderd uitgescheiden salicylzuur en daardoor een verandering in de invloed in capaciteitsbeperkt metabolisme. Ook farmacokinetische interacties met enige stoffen waarvan verwacht kan worden dat ze de salicylaat-klaring zouden beïnvloeden wordt besproken. Benzoezuur lijkt het metabolisme van salicylzuur zowel competitief als non-competitief te remmen. Probencid beïnvloedt de renale excretie én het metabolisme van salicylzuur negatief. D-glucuronolactone heeft geen duidelijke invloed, maar neigt tot
remming van de eliminatie, terwijl het tegenovergestelde verwacht werd.

Bij chronische medicatie blijkt een verdubbeling van de dosis van 1.5 tot 3.0 g per dag te leiden tot een 4- à 5-voudige toename van de gemiddelde plateauconcentratie, hetgeen in overeenstemming is met de beschouwing in hoofdstuk 15. Ook worden een aantal biofarmaceutische aspecten beschreven. Bij chronische medicatie met gelijke doseringen blijkt Enterosalicyl® (enteric coated natriumsalicylaat) aanzienlijk hogere plateauconcentraties te geven dan een oplossing van natriumsalicylaat. Het verschil is hoogst waarschijnlijk gelegen in de aanwezigheid van natriumbicarbonaat in de drank.

Na enkelvoudige dosering is de resorptie van salicylzuur uit de oplossing veel sneller dan uit Enterosalicyl® tabletten. In acute situaties zijn ook twee acetylsalicylzuur-preparaten vergeleken: Ascal®, het wateroplosbare calciumacetylsalicylaat en Rhonal®, gecoate microkristallen. Het wateroplosbare Ascal® wordt sneller geresorbeerd en in het algemeen wordt gesteld dat alleen wateroplosbare acetylsalicylzuur-preparaten de mogelijkheid bieden gebruik te maken van de specifieke analgetische activiteit in vergelijking met salicylzuur. Tenslotte worden een aantal experimenten beschreven ter vergelijking van salipyrine, een moleculaire verbinding van salicylzuur en fenazon, met natriumsalicylaat en fenazon afzonderlijk in oplossing. Vanuit een farmacokinetisch oogpunt is salipyrine geen optimale doseringsvorm, noch van salicylzuur, noch van fenazon.
Bij de voltooiing van dit proefschrift wil ik mijn erkentelijkheid betuigen aan allen die op enigerlei wijze een bijdrage hebben geleverd aan de onderzoekingen.

Voor het creëren van de vriendschappelijke en stimulerende sfeer, waarin het onderzoek kon plaatsvinden, dank ik alle medewerkers van de afdeling farmacologie.

Onmisbare hulp verleenden: Mej. F. Vrancx, die met zorg en met een gezonde kritische geest talloze plasma- en urinemonsters analyseerde; Mevr. C. Verweij en Mej. S. Torn, die de bloedmonsters afnamen bij vele vrijwilligers; Mevr. E. Klok, die een wezenlijke bijdrage leverde aan de analyse van plasmacurves en aan vele farmacokinetische modelberekeningen; Mej. M. Janssen, die op uittrekkende wijze het vele typewerk verzorgde en Mej. J. Hurkmans, die altijd tot hulp bereid was.

Verder dank ik: Dr. R. de Graaf voor waardevolle adviezen bij de toepassing van het curve-fitting programma Farmfit en bij simulaties van kinetische modellen; Drs. A. Tomey voor hulp bij een aantal berekeningen; de leden en ex-leden van de werkgroep Farmacokinetiek (Prof. Dr. D. Breimer, Drs. H. Fleuren, Drs. H. Ketelaars, Drs. J. van Kordelaar, Dr. T. Vree, Drs. T. Yih) voor suggesties en discussies.

CURRICULUM VITAE


Uit gezamenlijk onderzoek zijn de volgende publicaties voortgekomen:


CAM van Ginneken and J M van Rossum Nonlinear pharmacokinetics single and chronic administration *Proc Symp Pharmacokin and Drug effects Acta Pharmaceut Suec* 11, 642 (1974)

P Th Henderson, T B Vree, CAM van Ginneken and J M van Rossum Activation energies of α-C-oxidation of N-alkyl substituted amphetamines by rat liver microsomes *Stereochemistry and deuterium isotope effects Xenobiotica* 4, 121 (1974)


CAM van Ginneken and J M van Rossum Non-linear clearance *Pharm Weekblad* 110, 1253 (1975)

CAM van Ginneken, H L J M Fleuren and J M van Rossum Clearance of analgesics in man *Pharm Weekblad* 110, 1220 (1975)


CAM van Ginneken Middelen ter behandeling van Arthritis urica en hyperuricemie *Ibid*, p 571

CAM van Ginneken en J M van Rossum Antipyretische analgetica *Ibid*, p 541

STELLINGEN
Op theoretische zowel als praktische gronden verdient de klaringsfunctie de voorkeur boven de in de literatuur vrijwel uitsluitend gebruikte snelheidsconstant als de basale parameter voor de eliminatie van farmaca.

II

Hoewel de auteurs zelf de mogelijkheid van "dose-dependent kinetics" aanduiden, gaan de berekeningen van Lund et al. betreffende de biologische beschikbaarheid en de te verwachten plateauspiegels van fenytoïne geheel voorbij aan de implicaties van een dergelijk fenomeen en is hun analyse op beide gebieden volstrekt onjuist.


III

Bepaling van het totale lichaamswater volgens de methode van Huckabee kan gemakkelijk tot verkeerde uitkomsten leiden.


IV

De verklaring van Botha voor de verlaagde salicylaatspiegels bij gelijktijdig gebruik van acetosal en antacida, nl. verminderde absorptie van acetosal, is erg onwaarschijnlijk.


V

Hoewel capillaire kolommen in de gaschromatografie een waardevolle uitbreiding geven van de mogelijkheden voor kwalitatieve analyse van biologische monsters, lijkt hun waarde voor kwantitatieve farmacokinetische studies vooralsnog beperkt.

VI

De wijze waarop Costall en Naylor de door apomorphine veroorzaakte stereotypie kwantificeren, zal onveranderlijk een dosisafhankelijkheid van deze stereotypie laten zien. De curves die zij verkrijgen door de intensiteit van de stereotypie uit te zetten tegen de logarithme van de dosis mogen echter volstrekt niet beschouwd worden als dosis-werkingscurves zoals die in de moleculaire farmacologie bekend zijn.

VII

De opmerkingen van Wagner over een artikel van Van Ginneken et al. kunnen slechts verklaard worden door aan te nemen dat hij het betreffende artikel niet helemaal gelezen of helemaal niet begrepen heeft.


VIII

Duur en intensiteit van verslaving aan narcotische analgetica zijn mede afhankelijk van farmacokinetische factoren.

IX

Nu naast de propyl- en methylhomologen van de natuurlijke cannabinoiden in cannabis onverwacht ook butylhomologen zijn aangetroffen moet men verwachten dat nog verschillende andere homologe reeksen zullen worden gevonden.


X

Wie in zijn omgeving amateur-tuiniers gebruik ziet maken van herbiciden, kan met vreugde constateren dat het spreekwoord: "Onkruid vergaat niet", zowel letterlijk als figuurlijk, een kern van waarheid bevat.

XI

De hausse in nostalgische kinderboeken valt moeilijk te rijmen met de huidige emancipatiebeweging.

C.A.M. VAN GINNEKEN

NIJMEGEN, 8 SEPTEMBER 1976
Implications

Clinical-pharmacological

and its

in man

study

Kinetic

Fundamental