
jan h.g. jonkman
thiazinamium
methylsulphate,
bioanalysis and pharmacokinetics

STELLINGEN

I

Bij het uitvoeren van farmacokinetisch onderzoek is een gedegen kennis van de fundamentele aspecten van de bioanalyse onontbeerlijk.

II

Farmacokinetische parameters verkregen met behulp van computerprogramma's dienen met zorg te worden geïnterpreteerd.

III

De benaming „stikstof-detector" (= „nitrogen detector") voor een gaschromatografische detector, berustend op het principe van alkali vlam ionisatie, doet ten onrechte vermoeden dat deze detector uitsluitend gevoelig is voor stikstof bevattende verbindingen.

Maier-Bode, H. en M. Riedmann (1975), in: *Residue Reviews,* Vol. 54 (F. A. Gunther en J. Davies Gunther, Eds.), Springer Verlag, New York, Berlin.

IV

Het optreden van een plateauvormige plasmaconcentratie-tijdcurve na orale toediening van thiazinamium methylsulfaat kan bij chronische medicatie eventuele variaties in de biologische beschikbaarheid voor een belangrijk deel compenseren.

Dit proefschrift.

V

De opvatting dat geneesmiddelen na rectale toediening de algemene circulatie kunnen bereiken zonder voorafgaande leverpassage is in zijn algemeenheid niet juist.

Dit proefschrift.

VI

Bij het geven van een verklaring voor de discrepantie tussen de uitkomsten van hun eigen experimenten en die, welke verkregen waren door Hansson en Schmitterlöw, hebben Huang en medewerkers onvoldoende aandacht besteed aan de biofarmaceutische aspecten van hun experimenten.

Huang, C. L., J. A. Yeh en S. Y. Hsu (1970), *J. Pharm. Sci. 59,* 772.
Hansson, E. en C. G. Schmitterlöw (1961), *Arch. Int. Pharmacodyn. 131,* 309.

VII

Gezien de zeer geringe mate waarin thiazinamium methylsulfaat de bloed-hersenbarrière passeert, is het niet waarschijnlijk dat beïnvloeding van centraal-nerveuze functies een aanleiding vormt om dit geneesmiddel bij aflevering te voorzien van de waarschuwing „Dit geneesmiddel kan de rijvaardigheid beïnvloeden".

„Herziene lijst betreffende geneesmiddelen en verkeersveiligheid behorende bij het zwaarwegende advies van K.N.M.G. en K.N.M.P." (1973).

VIII

Teneinde verschraling van de universitair-farmaceutische vorming der Nederlandse apothekers te voorkomen, dient men bij de benoeming van docenten aan de Subfaculteiten Farmacie er voldoende zorg voor te dragen dat een goede interactie tussen de algemene farmaceutische praktijk en de opleiding tot apotheker wordt gewaarborgd.

IX

Een grotere kennis omtrent de werking van geneesmiddelen bij de gebruiker ervan kan leiden tot een meer selectieve geneesmiddelenconsumptie en een grotere „patient compliance".

X

Het in der loop der jaren gegroeide gebruik van promovendi in de Subfaculteiten Farmacie om in de vorm van een stelling, behorend bij hun proefschrift, kritiek te uiten op de Nederlandse Farmacopee zonder deze kritiek gemotiveerd onder de aandacht van de Farmacopee-commissie te brengen, moet als weinig opbouwend worden beschouwd.

XI

Door tranquillizers en hypnotica uitsluitend in de vorm van suppositoria beschikbaar te stellen aan diegenen, die aan deze medicamenten zijn verslaafd, kan overconsumptie worden vermeden.

XII

Uit het feit, dat de ziekenfondsen in het algemeen geen vergoeding geven voor een pruik voor mannen, maar wel voor vrouwen, blijkt een miskenning van de ijdelheid van de man.

XIII

Tegen de indringende reclame van de farmaceutische industrie voor hun geneeskrachtige synthetische producten is geen kruid gewassen.

XIV

Uit het feit, dat „auto" vaak één der eerste woorden is in de vocabulaire van een kind, blijkt de belangrijke plaats die dit vervoermiddel in de huidige maatschappij inneemt.

J. H. G. JONKMAN

Groningen, 20 juni 1977.

Thiazinamium methylsulphate, bioanalysis and pharmacokinetics

A study including biopharmaceutics, biotransformation and autoradiography

Omslag: Microscopische opname van thiazinamium jodide tijdens het uitkristalliseren.
Opname: Dr. Jean H. B. Kleikamp.

Rijksuniversiteit te Groningen

Thiazinamium methylsulphate, bioanalysis and pharmacokinetics

A study including biopharmaceutics, biotransformation and autoradiography

PROEFSCHRIFT

ter verkrijging van het doctoraat in de wiskunde
en natuurwetenschappen aan de Rijksuniversiteit
te Groningen op gezag van de Rector Magnificus
Dr. M. J. Janssen in het openbaar te verdedigen op
maandag 20 juni 1977
des namiddags te 4.00 uur

door

Jan Hasker Gerhardus Jonkman

geboren te Oosterwolde (Fr.)

PROMOTORES:	PROF. DR. J. S. FABER
	DR. R. A. DE ZEEUW
COREFERENTEN:	PROF. DR. D. A. DOORNBOS
	PROF. DR. N. G. M. ORIE
	DR. E. VAN DER KLEIJN

Aan mijn ouders,
voor Ineke en Marieke

Dankwoord

Graag wil ik op deze plaats mijn erkentelijkheid betuigen aan allen, die ertoe hebben bijgedragen dat dit proefschrift tot stand kon komen.
Alle medewerkers van de Farmaceutische Laboratoria en in het bijzonder van het Laboratorium voor Farmaceutische en Analytische Chemie ben ik veel dank verschuldigd voor hun altijd aanwezige bereidheid tot medewerking.
Bijzonder veel dank ben ik verschuldigd aan Prof. Dr. J. S. Faber, die het onderzoek initieerde door de contacten met de Kliniek voor Inwendige Ziekten, afdeling Longziekten van het Academisch Ziekenhuis tot stand te brengen. Zijn bereidwilligheid om mij in de gelegenheid te stellen mij in allerlei facetten van het onderzoek nader te verdiepen, soms ook in andere laboratoria, en het enthousiasme waarmee mogelijkheden werden gecreëerd om met buitenlandse collegae van gedachten te wisselen, vervult mij met grote dankbaarheid. De vele discussies, veelal over farmaceutische aangelegenheden, heb ik als zeer vormend ervaren.
Veel erkentelijkheid ben ik verschuldigd aan Dr. R. A. de Zeeuw voor de enthousiaste wijze, waarop hij zich inzette om de werkgroep goed te laten functioneren en voor de zeer vele uren, die hij uittrok voor het bespreken van de tekst voor dit proefschrift en van andere publicaties. Voor zijn stimulerende hulp en waardevolle inspiraties ben ik zeer dankbaar. Nimmer deed ik vergeefs een beroep op hem.
Prof. Dr. D. A. Doornbos dank ik voor zijn opbouwende suggesties betreffende dit proefschrift en de minutieuze wijze waarop hij het manuscript heeft doorgelezen.
Prof. Dr. N. G. M. Orie dank ik hartelijk voor het grote vertrouwen, dat hij in mij stelde, voor zijn kritische beoordeling van de voortgang van het onderzoek en voor de voortdurende steun en medewerking ter continuering van het onderzoek. Zijn vermogen zich te verdiepen in specifieke farmaceutische problemen heeft mij met ontzag vervuld. De korte opmerkingen van levensbeschouwelijke aard zal ik niet spoedig vergeten.
Drs. L. E. van Bork ben ik zeer erkentelijk voor haar niet aflatend enthousiasme en grote toewijding bij het onderzoek. De voortdurende bereidheid tot medewerking heeft op mij grote indruk gemaakt. Het stemt mij gelukkig dat uit deze intensieve samenwerking een hartelijke vriendschap is gegroeid.

Met grote erkentelijkheid vermeld ik tevens de bijdragen die J.Wijsbeek aan dit onderzoek heeft geleverd. Zijn grote vakkennis en bekwaamheid, zijn groot waarnemingsvermogen, kritisch inzicht en loyaliteit hebben in belangrijke mate bijgedragen aan dit onderzoek. Mevr. S. Hollenbeek Brouwer-de Boer en Mevr. A. S. Bolhuis-de Vries wil ik graag bedanken voor de prettige en accurate hulp bij de analyse van de talrijke monsters, en Mevr. A. S. Bolhuis-de Vries tevens voor haar spontaan aangeboden hulp bij het vele typewerk. Ook de bijvakstudenten die aan dit onderzoek meewerkten wil ik graag bedanken: Drs. J. E. Greving, (die later als collega zijn medewerking verleende bij het uitrekenen van de farmacokinetische parameters), Drs. D. J. A. Crommelin, Drs. H. G. P. van Asperen, Drs. R. E. M. van Gorp, Drs. L. H. A. J. Arts en Drs. C. Neef. Verder ben ik veel dank verschuldigd aan: Mevr. Drs. G. Venema-Verdenius voor de hulp bij het literatuuronderzoek; Drs. F. J. W. van Mansvelt voor hulp, die hij diverse malen gaf; Drs. H. G. M. Westenberg, voor de zeer collegiale wijze waarop hij meewerkte aan de vele experimenten in Nijmegen.
Dr. E. van der Kleijn, St. Radboud Ziekenhuis, afd. Klinische Farmacie, Katholieke Universiteit, Nijmegen, ben ik zeer dankbaar voor de mogelijkheden die hij creëerde om een aantal onderzoekingen van gemeenschappelijke interesse uit te voeren op zijn afdeling en voor zijn waardevolle adviezen. De medewerkers van het Centraal Dierenlaboratorium aldaar, de heren T. H. M. Arts, A. J. Peters en P. G. H. Philipsen dank ik voor hun medewerking aan de farmacokinetische experimenten en de heren G. J. F. Grutters en N. V. M. Rijntjes voor hun hulp bij de uitvoering van de autoradiografie-experimenten. De heer J. Konings, afdeling Medische Illustraties aldaar, was zo vriendelijk op de hem zo eigen wijze een aantal illustraties te verzorgen en Dr. R. de Graaf gaf mathematische adviezen.
Drs. A. M. Soeterboek en de heer M. van Thiel (Diaconessenhuis, Eindhoven) assisteerden op punctuele wijze bij het onderzoek met gal-fistel patienten. Zeer erkentelijk ben ik voor de buitengewoon plezierige samenwerking met Drs. H. L. M. Cox, Laboratorium der Nederlandse Apothekers, Den Haag. Zijn deskundige adviezen heb ik zeer gewaardeerd. Prof. Dr. D. D. Breimer, Vakgroep Farmacologie en Farmacotherapie der Subfaculteit Farmacie, Rijksuniversiteit Leiden, wil ik graag bedanken voor de vele waardevolle suggesties die ik van hem mocht ontvangen en voor zijn bereidheid een groot deel van het manuscript van dit proefschrift door te nemen. Prof. Dr. P. H. J. Hoedemaeker, Pathologisch Anatomisch Laboratorium, Rijksuniversiteit Groningen was zo vriendelijk om te helpen bij de evaluatie van de autoradiografie-experimenten. Onmisbare hulp werd verleend door diverse collegae werkzaam aan deze universiteit nl.: Dr. D. K. F. Meijer, Farmacologisch Laboratorium, bij de uitvoering van levertransport- en galexcretie-experimenten in de rat; door Dr. W. Vaalburg en Dr. S. Reiffers, Centraal Isotopenlaboratorium bij de syntheses en Drs. J. J. Pratt en Drs. A. Versluis, Centraal Isotopenlaboratorium, bij de radiochemische experimenten.

Prof. Dr. G. Schill, Laboratorium voor Farmaceutisch-Analytische Chemie, Universiteit Uppsala, Zweden en zijn medewerkers (vooral B. Fransson) ben ik zeer erkentelijk voor de enthousiaste wijze waarop zij mij hebben ingewerkt in de problematiek van ionpaar-extractie en Prof. Dr. M. Rowland, Farmaceutisch Laboratorium, Universiteit Manchester, Engeland en zijn medewerkers (speciaal Dr. B. Calvert en Dr. L. Aaron) voor hun medewerking bij het uitrekenen van de farmacokinetische parameters m.b.v. het NONLIN programma.
Voor de prettige samenwerking wil ik graag bedanken: Mevr. R. Hemmer-Kuiper voor het vele nauwgezette typewerk dat zij verzorgde; Mevr. G. Hayer-Herder, die bijna alle illustraties maakte; de heer J. M. Verhoog en Mevr. S. Lewis voor de correctie van de Engelse tekst; de medewerkers van de Centrale Fotodienst (speciaal de heren P. H. Smid, A. Luttmer, W. Haaima en G. Tammeling); de medewerkers van Wolters-Noordhoff Grafische Bedrijven (vooral de heren J. Swart, W. Antoons en L. Kemme). De medewerkers van SPECIA (de heren E. Etrillard, M. Bentejac, M. van den Berghe en R. Compier) ben ik zeer erkentelijk voor hun belangstelling voor het onderzoek, voor het beschikbaar stellen van gegevens, referentiestoffen, radioactieve verbindingen *etc.* Dr. J. H. B. Kleikamp, apotheker en chemicus te Hoorn wil ik graag hartelijk bedanken omdat hij onmiddellijk bereid was zijn kennis en vaardigheid van de microscopische kristalfotografie beschikbaar te stellen, hetgeen resulteerde in het omslag van dit proefschrift.
De vele patienten en vrijwilligers, die participeerden in dit onderzoek ben ik veel dank verschuldigd. Hun grote geduld en nauwgezette medewerking, soms gedurende enkele dagen, heb ik zeer bewonderd.

Graag wil ik mijn erkentelijkheid tot uiting brengen jegens mijn ouders, die mij in staat hebben gesteld een universitaire opleiding te volgen.

Tenslotte wil ik mijn vrouw Ineke hartelijk bedanken voor haar grote bijdrage in dit proefschrift. Zij was het, die mij ertoe aanzette dit onderzoek uit te voeren om zo mijn studie in de farmacie af te ronden. Zij was een onmisbare steun tijdens het onderzoek. Haar daadwerkelijke hulp kwam tot uiting in de consciëntieuze wijze, waarop zij de drukproeven corrigeerde. Haar raadgevingen waren steeds zeer stimulerend.

Contents

PART II
BIOANALYSIS

Voor de uitvoering van dit onderzoek werd financiële steun verkregen van de Gezondheidsorganisatie T.N.O. (subsidie no. G.O. 741-62) en van het Nederlands Astma Fonds.

De uitgave van dit proefschrift werd mede mogelijk gemaakt door een financiële bijdrage van het Nederlands Astma Fonds.

General introduction

There are several groups of drugs which can be applied in the treatment of pathophysiological airway obstructions, such as parasympatholytics, sympathomimetics, xanthine-derivatives, antihistaminic and antiserotonine drugs, corticosteroids and cromoglycate. Among these, drugs with anticholinergic and antihistaminic properties are administered in the treatment of certain types of obstructive lungdiseases.

One of these drugs is thiazinamium methylsulphate, a phenothiazine derivative with a quaternary ammonium group in the molecule. It has been widely used in the Netherlands for the past two decades, in two dosage forms: intramuscular injection and coated tablets for oral use. In general, a marked bronchodilatation was observed after an intramuscular injection, but some clinical observations gave rise to doubt about the efficacy of oral thiazinamium therapy. Unfortunately, in literature there is no information about adequate methods for the bioanalysis of thiazinamium methylsulphate, nor about the bioavailability of the drug.

For these reasons we were contacted by Prof. Dr. N. G. M. Orie and Drs. L. E. van Bork (Pulmonary Division of the Medical Department, University of Groningen). They asked us to develop a method for the determination of plasma concentrations of thiazinamium methylsulphate in order to study the bioavailability of the drug after oral administration.

After we succeeded in determining nanogram concentrations of the drug in body fluids, we decided to undertake a comparative study of the pharmacokinetics (absorption, distribution, metabolism and elimination) of this drug after different routes of administration and began an investigation to see if a correlation exists between the plasma concentration of the drug and its effect (lung function) and side-effect (heart rate).

The absolute bioavailability of an intramuscular injection was determined as compared to an intravenous injection of the same dose. Factors influencing this bioavailability (site of injection, muscle contraction) were also studied. Moreover the influence of the dose on the bioavailability was investigated by intramuscularly injecting the same patient with two different doses.

The main part of our investigations concerned the bioavailability of two oral doses as compared to an intramuscular injection and a study of interindividual and intraindividual variations of the bioavailability.

We also examined the bioavailability of the drug after rectal administration, using different suppository bases and different counter ions for the thiazinamium cation.
Most of these studies were carried out in patients suffering from generalized obstructive lung diseases.
In addition we did some investigations in man and in animals to obtain a more detailed image of a number of phenomena (biliary excretion, "first pass effect", distribution by means of whole body autoradiography *etc.*).
This thesis is divided into three parts.
In Part I, entitled "A profile of the drug under study", a description will be found of some properties of thiazinamium methylsulphate. These properties are related to the bioanalysis and pharmacokinetics of the drug.
In Part II, called "Bioanalysis" a general introduction is given about ion pair extraction as an isolation method in the analysis of drugs and drug metabolites in body fluids. It includes a large number of examples from literature. In this part a new method for the quantitative analysis of thiazinamium cations in plasma, urine, bile and saliva (using an ion pair extraction method) will also be discussed. Further a new method for the quantitative analysis of the metabolite thiazinamium sulphoxide cation in urine and bile will be discussed; this method is based on column chromatography followed by thin-layer chromatography with densitometry.
In Part III, called "Pharmacokinetics" the biopharmaceutical and pharmacokinetic studies, including metabolism and excretion are described.
The relation between plasma concentration of thiazinamium cations and the heart rate in man is dealt with in this part too. A study using whole body autoradiography in monkey and mouse gives an idea of the distribution of ^{35}S-thiazinamium methylsulphate and ^{35}S-promethazine hydrochloride.
The clinical aspects of this study and the relevance of the results for medical practice form the subject of the thesis of L. E. VAN BORK entitled "Thiazinamium methylsulphate, pharmacodynamics and clinical application".

Part I

A profile of the drug under study

A profile of the drug under study

Contents

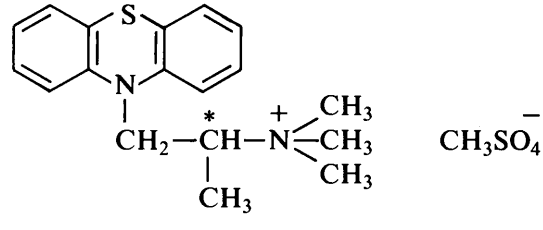
Some properties of thiazinamium methylsulphate will be dealt with, which are related to its bioanalysis and pharmacokinetics and which are relevant to the matters discussed in this thesis.
Some analytical chemical, physicochemical and pharmacological data, partly gathered from literature, partly determined in our own studies, will be mentioned in short terms. The data of our own finding were obtained with thiazinamium methylsulphate, batch no. 5229, SPECIA, Rhône Poulenc, Paris, France. Most of the data were obtained in our own experiments, unless stated otherwise.

1. Description

1.1. Name, formula, molecular weight

Thiazinamium methylsulphate is the methylsulphate of
- 10-(2-trimethylammonium-propyl) phenothiazine ion (INFORMATORIUM MEDICAMENTORUM, 1976), also named
- trimethyl [1-methyl-2(phenothiazin-10-yl)ethyl] ammonium ion (CLARKE, 1975) or
- trimethyl [1-methyl-2-(10-phenothiazinyl)ethyl] ammonium ion, or
- N-[β-(10-phenothiazinyl)propyl] trimethylammonium ion (both MERCK INDEX, 1968) or
- (phenothiazinyl-10)-1-propyl-2-trimethylammonium ion (MULTERGAN®, *Note Technique*, 1961).

The drug was developed by SPECIA, Rhône Poulenc and received the number 3554 R.P.; it was synthesized by R. DUCROT, PH. DECOURT and J. FOURNEL (British patent 641.452; 1950).
The most commonly used trade name is Multergan®.
Other trade names are: Padisal®, Multezin® and Valan®.
The empirical formula is $C_{19}H_{26}N_2O_4S_2$.
The molecular weight is: 410.55.
The structural formula is:



1.2. Appearance, colour, odour, taste

Thiazinamium methylsulphate occurs as a white crystalline powder. Its colour slightly changes to grey, when exposured to light. It is non-hygroscopic. The drug is odourless, tastes extremely bitter. Applied to the tongue it causes a transitory benumbing sensation.

2. Physical properties

2.1. Infrared spectrum (I.R.)

The I.R. spectrum of thiazinamium methylsulphate was obtained from a pellet made by dispersing 18 mg of the drug in 143 mg of potassium bromide. The spectrum is shown in Fig. 1. It was measured by us on a Beckman Acculab-2 Infrared Spectrophotometer.

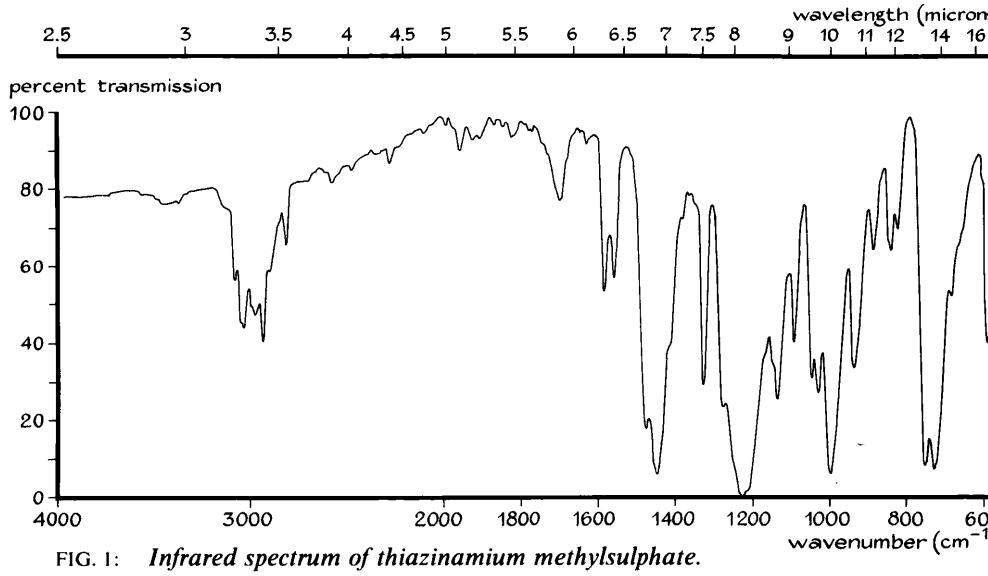


FIG. 1: *Infrared spectrum of thiazinamium methylsulphate.*

2.2. Nuclear magnetic resonance spectrum (N.M.R.)

We have recorded a N.M.R. spectrum (Fig. 2) of an almost saturated solution of thiazinamium methylsulphate in tetradeuteromethanol to

which 5% tetramethylsilane was added as an internal reference. A Hitachi Perkin-Elmer R-24 High Resolution N.M.R. Spectrometer was used.

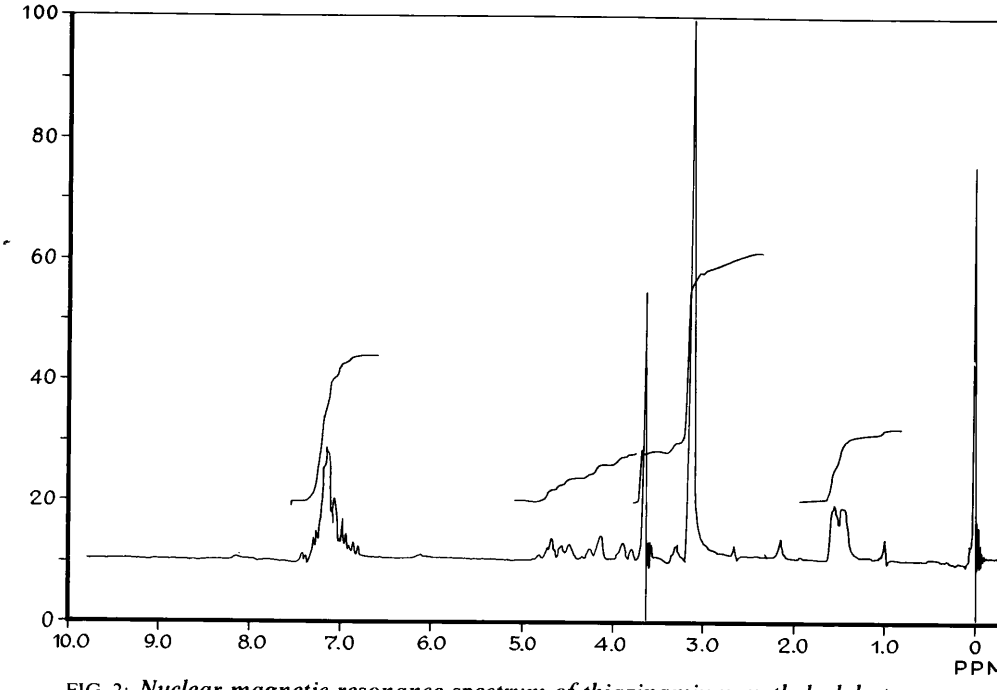


FIG. 2: *Nuclear magnetic resonance spectrum of thiazinamium methylsulphate.*

The following N.M.R. proton spectral assignments have been made.

Chemical shift (p.p.m. from tetramethylsilane)		Number of protons	Functional group
1.40–1.63	doublet	3	$-\overset{|}{\underset{|}{C}}-CH_3$
3.13	singlet	9	$-\overset{CH_3}{\underset{CH_3}{\overset{|}{\underset{|}{N^+}}}}-CH_3$
3.66	singlet	3	$-CH_3$ (from $CH_3SO_4^-$)
3.80–4.80	-	3	$-CH_2-\overset{|}{CH}-$
6.80–7.60	-	8	aromatic

Singlets at 1.00, 4.30 and 4.67 p.p.m. were caused by the solvent.

2.3. Ultra violet spectra (U.V.)

We recorded an U.V. spectrum of thiazinamium methylsulphate in distilled water (pH = 5.9) in 0.1 n hydrochloric acid and in ethanol 100% (all solutions appr. 20 µg/ml) on an Unicam SP 800 Ultra Violet Spectrophotometer. The spectra showed to have the same form; one of them (aqueous solution, pH = 5.9) is shown in Fig. 3.

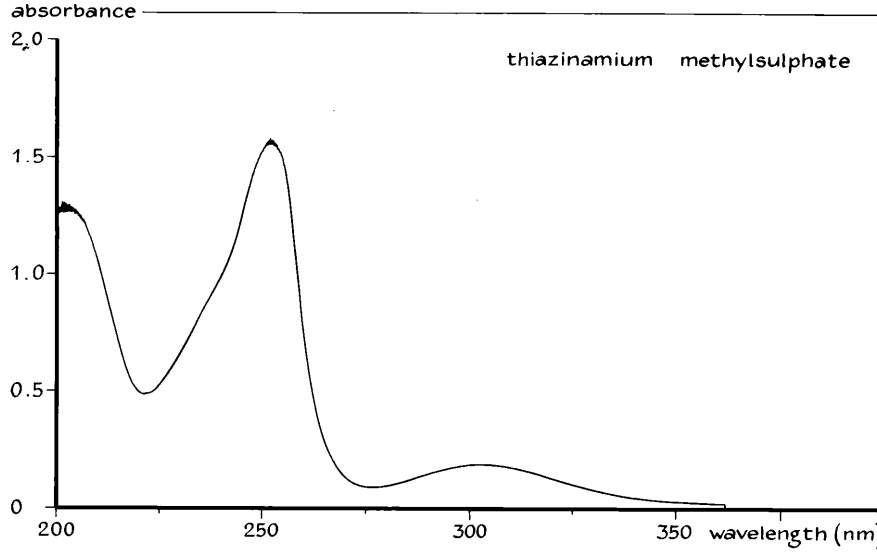


FIG. 3: *Ultra violet spectrum of thiazinamium methylsulphate in water of pH = 5.9.*

The values for the wavelength with maximum absorption, the specific extinction ($E^{1\%}_{1\,cm}$) and the molecular extinction coefficients (ε) are given in Table 1.

TABLE 1: *Wavelengths with maximum absorption* (λ), *the specific extinction* ($E^{1\%}_{1cm}$) *and the molecular extinction coefficients* (ε) *for thiazinamium methylsulphate in ultra violet light.*

	λ (nm)	$E^{1\%}_{1\,cm}$	ε (dm³/mol × cm)	λ (nm)	$E^{1\%}_{1cm}$	ε (dm³/mol × cm)
water, pH = 5.9	252	730	29970	303	81	3325
water, pH = 1.0	252	716	29395	303	75	3079
ethanol 100%	253	716	29395	305	72	2956

2.4. Mass spectrum

The mass spectrum of thiazinamium methylsulphate is shown in Fig. 4. It was recorded in our laboratory under the following conditions of measurement:

Apparatus	:	Finnigan G.C.-M.S. 3300
Sensitivity	:	10^{-6} A/V
Electron multiplier voltage	:	1.5 kV
Mass range	:	30–500
Scan control	:	0.1 scan/sec.
Emmission current	:	30 V
Electr. energy	:	70 eV
Solid probe	:	400° C
Analyzer temperature	:	80° C

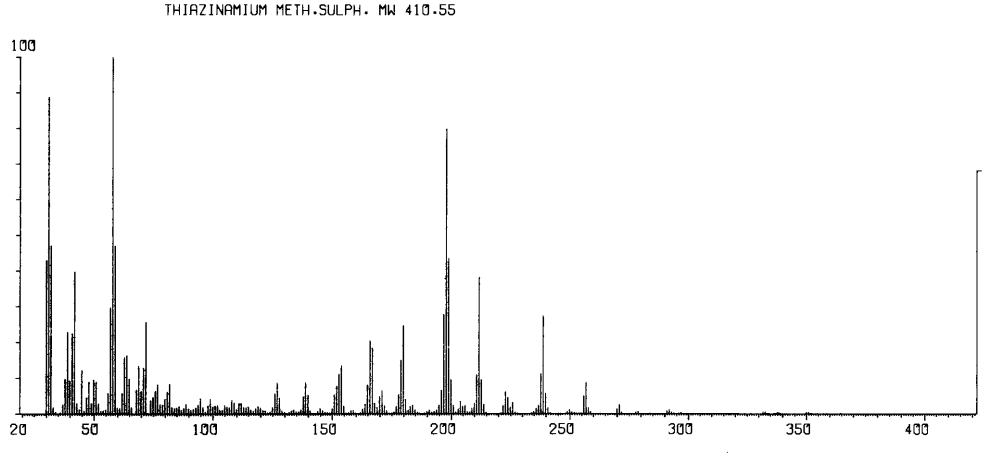


FIG. 4: *Mass spectrum of thiazinamium methylsulphate.*

2.5. Melting range

The melting range, determined in accordance with the method of the DUTCH PHARMACOPOEIA ED VII (= European pharmacopoeia ED I) was recorded to be 206°–210° C, under decomposition.

2.6. Solubility

Solubility data were obtained by us using a method, modified according to the U.S.P. XIX (1975) method for the determination of the "Approximate Solubility".

Procedure: First a preliminary estimation was performed by adding thiazinamium methylsulphate to the solvent until no further dissolution occurred. Then the definitive determination was performed as follows: 10.0 ml of the solvent was transferred to a glass tube of 50 ml capacity and placed in a water bath at 25° C. An amount of thiazinamium methylsulphate equal to the value found in the preliminary estimation was added to the solvent and the two were mixed. If necessary, more solute was added until an excess was seen at the bottom of the tube. Then the tube was vigorously shaken on a Vortex® mixer during 30 sec. at maximum velocity and put back in the water bath for 15 min. Then shaking was repeated, now during 30 sec. and again the tube was placed in the water bath for 15 min. Next a 2 ml sample was filtered through a G-4 glass filter and diluted as far as was necessary to allow spectrophotometric quantitation.
The spectrophotometric measurements were done at a wavelength of 252 nm. However some of the solvents themselves absorb light at this wavelength (benzene and the chlorinated hydrocarbons). In these instances the measurements were done at a wavelength of 303 nm (Apparatus: Zeiss PMQ II Spectrophotometer).

The following values were found:

Solvent	Solubility mg/ml	Solubility mol/l
water (pH = 5.9)	150.0	3.66×10^{-1}
methanol	201.0	4.90×10^{-1}
ethanol	17.5	4.27×10^{-2}
chloroform	10.2	2.49×10^{-2}
dichloromethane	6.3	1.54×10^{-2}
1,2-dichloroethane	2.3	5.61×10^{-3}
benzene	appr. 0.005	appr. 1.2×10^{-5}
n-hexane	appr. 0.001	appr. 2.4×10^{-6}
di-ethylether	< 0.001	< 2.44×10^{-6}

2.7. Dissociation constant; pH of an aqueous solution

Being a quaternary ammonium compound, thiazinamium methylsulphate is completely ionized at any pH.
A solution of 5% (w/v) in distilled water (pH = 6.1) has a pH of 5.9.

2.8. Distribution coefficients

The apparent distribution coefficient was determined at 25° C between n-octanol and an aqueous phase of pH = 1.2 (simulating the pH in the stomach) and also between n-octanol and an aqueous phase of pH = 7.4 (the physiological pH of the blood). The aqueous phase of pH = 1.2 contains diluted hydrochloric acid. The aqueous phase of pH = 7.4 was a phosphate buffer. The concentrations of the drug were measured spectrophotometrically; in the aqueous phase at 252 nm and in the organic phase at 254 nm.

Procedure: Thiazinamium methylsulphate was dissolved in 250 ml of the aqueous phase till ⅔ of saturation was reached. This solution was shaken with an identical quantity of n-octanol until equilibrium was reached (checked by taking samples at certain time intervals).

The solubilities in the two aqueous phases and in n-octanol and the distribution coefficients are shown in Table 2. The extremely low solubility in n-octanol as compared to the aqueous phases results in a distribution coefficient of almost zero in either case (data obtained from M. BENTEJAC (SPECIA, Rhône Poulenc, Paris, France).

TABLE 2: *Solubility of thiazinamium methylsulphate in n-octanol and in water of two different pH-values and the distribution coefficient between these two phases.*

	solubility in n-octanol		solubility in water		distribution coefficient n-octanol/water
	mg/ml	mol/l	mg/ml	mol/l	
pH = 1.2	0.9	2.2×10^{-3}	110	2.7×10^{-1}	0.008
pH = 7.4	0.9	2.2×10^{-3}	62	1.5×10^{-1}	0.015

2.9. Optical rotation

Thiazinamium methylsulphate is a racemic mixture.

3. Synthesis

Thiazinamium can be prepared by treating promethazine with a methylating agent (methyl iodide) according to Chemical Abstracts 45 (1951) 2511i (British patent 641.452 (1950)).

4. Methods of analysis

4.1. Elemental analysis

Element	% theoretically	% observed (mean; n = 2)*
C	55.58	55.5
H	6.38	6.5
N	6.82	6.8
O	15.59	15.4
S	15.62	15.5

* (Analysis performed at the Department for Chemistry, State University, Groningen).

4.2. Identification tests

Thiazinamium methylsulphate is not described in any pharmacopoeia. It responds to the general tests for phenothiazine derivatives. Some identification tests are given by the L.N.A. *Documentation System* (Laboratory of Dutch Pharmacists, The Hague), which uses the reagents and methods of the DUTCH PHARMACOPOEIA ED VI and VII. The applicable methods of identification in it are:

1. To 1 ml of a solution 1 = 1000 in distilled water add 2 ml of sulphuric acid: a slightly red colour appears; cool and add dropwise 0.1 n of potassium dichromate: the colour changes via dark red to green and further to brown.
2. To 5 ml of a solution of 1 = 1000 in distilled water add 2 ml of nitric acid: the colour changes via slightly red to dark red and then to colourless. The solution obtained gives no reaction for chloride nor for sulphate.

3. Add 2 ml of nitric acid to 50 mg of the substance. Dry carefully. Add 5 ml of water to the yellow residue and filter. The filtrate gives a positive reaction for sulphate.
4. Add 1 ml of sodium hydroxyde to 5 ml of a solution of 1 = 100 in distilled water: the solution will be clear (difference with promethazine hydrochloride and other tertiary phenothiazine derivatives).

The batch of thiazinamium methylsulphate (see Introduction) we used fulfilled the above mentioned tests.

4.3. Chromatographic analysis

4.3.1. Paper chromatography

CLARKE (1969 and 1975) has described a system for a paper chromatographic analysis. This method was originally devised by CURRY and POWELL (1954) and modified by CLARKE (1962).

4.3.2. Thin-layer chromatography

CLARKE (1969 and 1975) also described a thin-layer chromatographic system, which was originally devised by SUNSHINE (1963) but modified by CLARKE.
We developed a thin-layer chromatographic system which gives a separation between thiazinamium and the degradation and biotransformation product thiazinamium sulphoxide. This is discussed in Part II, Chapter 3, where also other references from literature will be quoted.

4.3.3. Gas chromatography

At the beginning of our studies no G.L.C. method for the determination of thiazinamium methylsulphate was available in literature. We therefore developed a new gas chromatographic method which is described in Part II, Chapter 2 (See also JONKMAN and others, 1975).

4.4. Direct spectrophotometric analysis

If no interfering species are present, the reported maximum at 252 nm in water can be used for quantitative analysis (see 2.3.). This method can also be used to assay solutions of thiazinamium methylsulphate that are used for injection (See Part III, Section 2.2. under "The dosage form"). These solutions contain 32.44 mg of thiazinamium methylsulphate per ml. After diluting these solutions 1 to 5000 they can be directly measured spectrophotometrically. The relative standard deviation of the method is 1.21% (n = 7).

4.5. Colorimetric analysis

A colorimetric method for the determination of thiazinamium methylsulphate in bulk substance and pharmaceutical dosage forms has been reported by DESSOUKY and others (1974), who used an ion pair extraction technique, with bromophenol blue as counter ion and monochlorobenzene as extraction solvent. The absorption was measured at 415 nm.
MEUNIER (1968) described a colorimetric method employing benzoparaquinone as reagent.

4.6. Electrochemical analysis

PATRIARCHE and LINGANE (1970) have described a coulometric method for the determination of thiazinamium methylsulphate.
An amperometric method has been used by BERAL and others (1965).

4.7. Titrimetric analysis

4.7.1. Cerimetric titration

The pure substance can be analyzed using the method of the L.N.A.-*Docu-*

mentation System (Laboratory of the Dutch Pharmacists, The Hague):

Dissolve 150 mg of thiazinamium methylsulphate in 30 ml of water, add 10 ml of diluted sulphuric acid and titrate with 0.05 n ceric sulphate until the appeared red colour has completely disappeared. 1 ml of 0.0500 n ceric sulphate = 10.25 mg of thiazinamium methylsulphate.

4.7.2. Amphimetric titration

We developed a new method for the determination of thiazinamium methylsulphate. It is based on the principle of amphimetric titrations. The method is selective for the drug; its decomposition product, thiazinamium sulphoxide is not determined in this way. The new method can be used for pure substance and dosage forms (*e.g.* injection solutions and coated tablets, as will be mentioned in Part III, Chapters 2 and 4). The principle of such a two phase titration system has been described by *e.g.* THOMIS and KOTIONIS (1956), JOHNSON and KING (1963), PELLERIN and others (1964), DEMAY (1966) and in the L.N.A.-MESSAGE (1969). An extensive review of amphimetry in general, with short reference to thiazinamium methylsulphate, has been written by DEMAY (1968).

Principle: an aqueous solution of thiazinamium methylsulphate is titrated with an aqueous solution of sodium dioctylsulphosuccinate (D.O.S.S.). Thiazinamium cation combines with dioctylsulphosuccinate to form an ion pair, which can be extracted from the aqueous layer by shaking with chloroform. As indicator dimethyl yellow is used, which forms a chloroform soluble ion pair with the first excess of D.O.S.S. At the end point, the colour of the chloroform layer changes from yellow to orange-red.

Reagents: acetic acid (30%), hydrochloric acid 0.5 n, chloroform, dimethyl yellow indicator solution; all as described in the DUTCH PHARMACOPOEIA ED VII. A 0.005 M D.O.S.S. solution is prepared by dissolving 2.5 g of sodium dioctylsulphosuccinate in 800 ml of boiling water. After cooling, water is added until a volume of 1000.0 ml is obtained. The strength of this titrant is determined against a standard reference of thiazinamium methylsulphate of known quality (at least 99.9% pure according to thin-layer chromatography): *c.* 410 mg of thiazinamium methylsulphate is accurately weighed and dissolved in distilled water. After addition of 2 ml of 0.5 n HCl distilled water is added until the volume is 100.0 ml. Next 3.00 ml of this solution is transferred to a volumetric flask of 300 ml, to which 7 ml of water, 1 ml of acetic acid (30%), 20 ml of chloroform and 2 drops of dimethyl yellow are added. The solution is then titrated as described under "Procedure".

The relative standard deviation of the method is 0.4% (n = 5).

Procedure:

1. Substance

0.03–0.05 mmol of thiazinamium methylsulphate is dissolved in a mixture of 10 ml of water and 1 ml of acetic acid 30%. Add 20 ml of chloroform and 2 drops of dimethyl yellow. Titrate with 0.005 M D.O.S.S. while mechanically shaking, until the colour of the chloroform layer changes from yellow to orange-red.

2. Dosage forms

a. Injections: 0.5 ml is directly titrated as described under "Substance".

b. Tablets: Dissolve 1 tablet after weighing, in a mixture of water and 2 ml of 0.5 n HCl and add water until a volume of 100.0 ml is obtained. Transfer 4.00 ml to a volumetric flask and continue as described under "Substance". (Note: Due to the insoluble excipiences in the tablet the solution will be turbid).

5. Protein binding

Binding of thiazinamium methylsulphate to plasma proteins was estimated by us using equilibrium dialysis. To this purpose plasma of a healthy volunteer was used, to which ^{35}S-labelled drug substance was added.

Procedure: Blood, obtained from venous puncture was defibrinated by stirring slowly and divided into two portions. To 25 ml of each portion 25 µl or 75 µl of an aqueous solution of ^{35}S-thiazinamium methylsulphate (specific activity 1.3 µCi/mg; see also Part III, Chapter 13) was added in such a way that concentrations were obtained which are comparable to those generally found in plasma after administration of a therapeutic dose of the drug (100 ng and 300 ng per ml plasma respectively). After standing 2 hours at 25° C with mixing now and then the blood was centrifugated for 15 min. at 6000 *g*. From the plasma obtained in this way 1.0 ml was transferred into a dialysis sac (Höchfelt, 8/32″ × 100′) which was closed and put into a glass tube containing 2.0 ml of Kreb's solution. The tube was closed and subsequently gently shaken for 17 hours in a water bath at 37° C. Then concentrations of the drug were measured in the plasma and in the Kreb's solution by means of liquid scintillation. To control if equilibration was complete the experiment was repeated, but now the drug substance was added to the Kreb's solution. Recovery of the procedure was checked to control if any drug was lost, due to irreversible adsorption to the dialysis sac.

The following data were obtained. Each value is the mean of three experiments.

concentration		plasma protein binding mean ± standard deviation	recovery mean ± standard deviation
(ng/ml)	(mol/l)	(%)	(%)
100	2.44×10^{-7}	82.3 ± 3.0	101.4 ± 5.0
300	7.31×10^{-7}	76.1 ± 3.8	91.6 ± 6.0

6. Pharmacological properties

6.1. Pharmacokinetic parameters

Because no sensitive method for analysis of thiazinamium methylsulphate in body fluids exists (see also Part II, Chapter 2), no pharmacokinetic parameters were known until now. In so far we investigated them, they are reported in Part III.

6.2. Pharmacodynamic properties

Thiazinamium methylsulphate features a prominent antihistaminic and anticholinergic activity, while a certain local anesthetic action can also be observed.

6.2.1. Antihistaminic activity

Thiazinamium methylsulphate has a very clear antihistaminic activity. This is probably due to the combination of the phenothiazine group with the aminoethane group in the side chain of the molecule. Its antihistaminic activity and that of its tertiary analogue promethazine hydrochloride (also known under the trade name PHENERGAN®) are listed in Table 3 (Data from DUCROT and DECOURT, 1950 b).

From this table the following can be concluded:

a. In the *"in vitro" antispasmodic activity* test thiazinamium methylsulphate is twice as effective as promethazine hydrochloride.
(Test: fragment of an isolated small intestine of a guinea pig in a solution of histamine; concentration of 0.1 mg/l).

b. In the *"Schaumann-test"* thiazinamium methylsulphate is 5 times more effective as promethazine hydrochloride. A dose of 1 mg/kg subcutaneously of either drug gives a protective action during 7 to 8 hours.
(Test: measurement of the protective action against a lethal bronchospasm in a guinea pig by an aerosol of histamine).

TABLE 3: *Antihistaminic activity of thiazinamium methylsulphate (quaternary ammonium compound) and its tertiary analogue promethazine hydrochloride, in various tests.*

Test	Thiazinamium methylsulphate	Promethazine hydrochloride
a. "In vitro" antispasmodic action		
Minimum effective concentration (mg/l)	0.025	0.050
b. "Schaumann-test"		
Minimum effective dose, subcutaneously (mg/kg)	0.1	0.50
c. "Bovet-Staub-test"		
Maximum tolerated number of lethal doses of histamine (after 0.6 mg/kg of the drug subcutaneously)	80	50
d. "Decourt-Bonvallet-test"		
Relative activity (see text)	4	1.8

c. In the *"Bovet-Staub-test"* thiazinamium methylsulphate is 1.6 times more effective than promethazine hydrochloride. (Test: measurement of the protective activity against a lethal bronchospasm induced by an intravenous dose of histamine; guinea pig; 0.6 mg/kg of the drug administered (subcutaneously)).

d. In the *"Decourt-Bonvallet-test"* thiazinamium methylsulphate is also the more effective drug (twice that of promethazine hydrochloride).
(Test: measurement of the elevation of the required amount of intravenously administered histamine to provoke a minimal action on vesical sphincter and on the respiration of histamine sensibilized animals (dogs). Drug injected I.V. 0.5 mg/kg).

The numbers in Table 3 are relative activities (N-dimethylaminoethyl-N-ethylaniline was used as standard).

From the above it can be concluded that thiazinamium methylsulphate has a prominent antihistaminic activity after intravenous or subcutaneous injection. This activity is even stronger than that of promethazine hydrochloride.

6.2.2. Anticholinergic activity

Probably due to the quaternary ammonium group, in combination with the aminoethane group, thiazinamium methylsulphate has a strongly marked anticholinergic activity. Its activity is compared with that of promethazine hydrochloride and atropine sulphate in Table 4. (Data from DUCROT and DECOURT, 1950 a).

TABLE 4: *Anticholinergic activity, in various tests, of thiazinamium methylsulphate (quaternary ammonium compound) and its tertiary analogue promethazine hydrochloride and atropine sulphate.*

Test	thiazinamium methylsulphate	promethazine hydrochloride	atropine sulphate
a. "in vitro" isolated intestine (action against 1×10^{-7} mol/l acetylcholine) Minimum effective dose mg/l.			
- guinea pig	0.25	2	0.002
- rabbit	0.25	1	0.005
b. "in vitro" isolated intestine (action against musculotropic action induced by barium chloride 200 mg/l) Minimum effective dose mg/l.			
- guinea pig	50	10	*
- rabbit	200	5	*
c. mydriatic action Minimum effective dose mg/kg.			
- mouse	5	5	*
d. antinicotinic action Minimum effective dose mg/kg.			
- dog	0.1	*	*

* No values reported in the reference quoted.

From this table the following can be concluded:

a. In the *"in vitro" anticholinergic* test on an isolated fragment of an intestine of a guinea pig thiazinamium methylsulphate is eight times more effective than promethazine hydrochloride, but 125 times less

effective than atropine sulphate. In the rabbit thiazinamium methylsulphate is 50 times less effective than atropine sulphate.
(Test: fragment of small intestine in a solution of 1×10^{-7} mol/l acetylcholine).

b. In the *"in vitro" anti-musculotropic activity* test thiazinamium methylsulphate is 5–40 times more effective than promethazine hydrochloride.
(Test: fragment of the isolated small intestine of a guinea pig; spasm provoked by a concentration of 200 mg/l of barium chloride).

c. In the test of the *atropine-like anticholinergic effect* thiazinamium methylsulphate is as effective as promethazine hydrochloride.
(Test: measurement of the mydriatic activity in the eye of a mouse after subcutaneous injection).

d. In the test of the *nicotine-like anticholinergic effect* thiazinamium methylsulphate provokes a paralysis of the vegetative ganglions (nicotine-type).

It can be concluded that thiazinamium methylsulphate has a rather strong anticholinergic activity after intravenous or subcutane injection; that this is more marked than that of promethazine hydrochloride, but less than that of atropine sulphate.

6.2.3. Other pharmacodynamic properties

Thiazinamium methylsulphate has a *local-anaesthetic* activity in a concentration of 5%. This is the same as for procaine hydrochloride. Promethazine hydrochloride is 5–10 times more effective in this respect. (MULTERGAN® 3554 R.P. (1961), *Note technique*).

6.3. Toxicity

In mice the following LD_{50} was measured:
- oral administration : 375 mg/kg
- subcutane administration : 250 mg/kg
- intravenous administration : 5 mg/kg

With other animals the LD_{50} after subcutane administration was lower (50–150 mg/kg in dog and rabbit).

Note the large difference between oral and intravenous injection (See also Part III, Chapter 4).

References

BERAL, H., L. MUREA, M. MADGEARU, E. CUCIUREANU and B. WERMESCHER (1965), *Pharm. Zentralhalle 104,* 231

CLARKE, E. G. C. (1962) in: *Methods of Forensic Sciences,* Vol. I, p. 31, F. Lundquist, Ed., Interscience Publisher, New York

CLARKE, E. G. C. (1969) in: *Isolation and identification of drugs,* Vol. 1, The Pharmaceutical Press, London

CLARKE, E. G. C. (1975) in: *Isolation and identification of drugs,* Vol. 2, The Pharmaceutical Press, London

CURRY, A. S. and H. POWELL (1954), *Nature 173,* 1143

DEMAY, D. (1966), *Cosmopharma 1,* 37

DEMAY, D. (1968) in: *Mises au point de Chimie Analytique Organique- pharmaceutique et Bromatologique,* J. A. Gautier and P. Malangeau, Eds., Mason et Cie, Paris

DESSOUKY, Y. M., B. A. MOUSA and H. M. NOUR EL-DIN (1974), *Pharmazie 29,* 579

DUCROT, R. and PH. DECOURT (1950a), *C. R. de la Soc. de Biol. 144,* 908

DUCROT, R. and PH. DECOURT (1950b), *C. R. de la Soc. de Biol. 144,* 911

DUTCH PHARMACOPOEIA ED. VII (1971)

INFORMATORIUM MEDICAMENTORUM (1976), H. de Ru, Ed., K.N.M.P., The Hague

JOHNSON, C. A. and R. J. KING (1963), *J. Pharm. Pharmacol. 15,* 584

JONKMAN, J. H. G., J. WIJSBEEK, S. HOLLENBEEK BROUWER-DE BOER, R. A. DE ZEEUW, L. E. VAN BORK and N. G. M. ORIE (1975), *J. Pharm. Pharmacol. 27,* 849

L.N.A., *Documentation System,* Laboratory of the Dutch Pharmacists, The Hague

L.N.A.-MESSAGE (1969), *Pharm. Weekblad 104,* 1247

MERCK INDEX, An Encyclopedia of Chemicals and Drugs (1968), P. J. Stecker, Ed., Rahway

MEUNIER, J. (1968), *Ann. Pharm. Franç. 26,* 25

MULTERGAN® 3554 R. P. (1961), *Note technique,* SPECIA, Rhône-Poulenc, Paris, France

PATRIARCHE, G. and J. J. LINGANE (1970), *Ann. Pharm. Franç. 28,* 511

PELLERIN, F., J. A. GAUTIER and D. DEMAY (1964), *Ann. Pharm. Franç. 22,* 495

THOMIS, G. N. and A. Z. KOTIONIS (1956), *Anal. Chim. Acta 14,* 11

SUNSHINE, I. (1963), *Am. J. Clin. Pathology 40,* 576

UNITED STATES PHARMACOPEIA XIX (1975), The United States Pharmacopeial Convention Inc., Rochville, Md.

Part II

Bioanalysis

1

Ion pair extraction as a method of isolation of drugs and drug metabolites in body fluids

Contents

Summary

For the determination of drugs and their metabolites in body fluids, the choice of isolation method is of great importance.
The ion pair extraction method has been known for several decades. In recent years, there has been an increase in the interest in this method as it appeared to be useful for isolating drugs and their metabolites from plasma, urine and bile. If circumstances are carefully chosen the selectivity of the method can be very high. With the help of modern instrumental separation methods, *e.g.* liquid chromatography and gas chromatography, extracts of high purity can be obtained, which enables detection of very small quantities (nanogram-range).
The general principles and a few theoretical aspects of the ion pair extraction method will now be discussed. Various applications and possibilities will be mentioned and a review based on literature will be given of the analysis of about 100 drugs by means of this method*.

* Contrary to other parts of this thesis, for practical reasons references will be numbered in this chapter.

Introduction

Organic substances can be extracted from aqueous solutions with an organic solvent which is not miscible with water, if the particular substance consists of uncharged molecules and if it has sufficient lipophilicity to dissolve in the organic solvent employed.
By means of this method it is possible to extract a large number of drugs from aqueous media, because many drugs are neutral, weak acids or weak bases. By bringing the medium to the correct pH these drugs can be made to assume an unionized form, after which extraction with a suitable organic solvent can be carried out. This form of liquid-liquid-extraction has seen large scale adoption in the quantitative analysis of drugs in biological materials ("bioanalysis").
However, not all drugs can be isolated in this way. Compounds, which are completely ionized at all pH-values, such as quaternary ammonium compounds, several sulphonic acids and carboxylic acids (*e.g.* amino acids), amino phenols and conjugates of metabolites (sulphates, glucuronides, *etc.*) can only be isolated after they have been derivatized or split up, or by means of an ion pair extraction method, as it is called.
The ion pair extraction method has been known for several decades. As early as 1931, HADDOCK and EVERS (37) described the method although they did not use the term ion pair extraction. They reported the isolation of strychnine in "Easton's Syrup" with chloride - a mixture of sodium chloride and hydrochloric acid - as counter ion and chloroform as extraction solvent. In other words, the extraction took place in an acid medium. This was a totally new development since until then it had been usual to extract alkaloids as bases from an alkaline medium. AUERBACH (2) was the first to describe the systematic use of this method in the determination of quaternary ammonium ions. Until then it was only possible to determine the strength of solutions of these desinfecting components on the basis of a "phenol coefficient".
AUERBACH described the formation of "coloured salts" of certain quaternary ammonium compounds with dibromothymolsulphophtalein (= bromothymol blue) and tetrabromophenolsulphophtalein (= bromophenol blue). These "coloured salts" could be easily extracted from an alkaline medium with 1,2-dichloroethane. One year later AUERBACH (3) reported that extraction is also possible with benzene. Although these "coloured salts" are less easy to dissolve in benzene, the latter liquid offers several practical advantages (*e.g.* better separation from the aqueous layer after shaking). These publications were rapidly followed by others, in which especially indicators from titrimetric analysis were employed as counter ions, which made such names popular as "acid dye method", "dye salt method" and "indicator method". After it became clear from publications in the early 1960-ies by SCHILL and colleagues (see *e.g.* 94), that other

counter ions can be used too, the term "ion pair extraction" was generally accepted (see *e.g.* 98).

1.1. General principles of the ion pair extraction method

In the past few years several hundred publications have appeared, in which the ion pair extraction method was described. Much work of a fundamental nature was done by SCHILL and his co-workers (University of Uppsala, Sweden) who since 1959 reported with great regularity the results of their investigations (from 1964 especially in *Acta Pharmaceutica Suecica*).
T. HIGUCHI *c.s.* also did fundamental research on ion pair extraction (38, 39, 40, 41). DIVATIA and BILES (21, 16) systematically investigated the influence which several parameters have on the distribution coefficient of alkylamines and quaternary ammonium compounds between two inmiscible phases.
Application of the ion pair extraction method to the analysis of drugs in several dosage forms was extensively studied by LEVINE and DOYLE (22, 23, 55, 56, 57, 58, 59). KRAUS (53) published a comprehensive study on evolution and application of ion pair formation in 1956. This author alone gives an appropriate definition of the term "ion pair".
Ion pairs, according to KRAUS, are "neutral species formed by electrostatic attraction between oppositely charged ions in solution and they are often sufficiently lipophilic to dissolve in non-aqueous solvents" (*l.c.*).
We ourselves, however, prefer the following description:
"An ion pair is an association-complex of two charged particles (ions), one of which often is of organic nature; because they neutralize each other's charge the ion pair, which has been formed from the two ions, has become more lipophilic in character. Ion pairs, then, are in general insoluble in an aqeous medium, but are easily soluble in such weakly polar organic solvents as chloroform, dichloromethane, 1,2-dichloroethane and some higher alcohols like pentanol". It remains a prerequisite, that the compound to be extracted is in a charged state or can be easily brought into this condition.
In the discussion of the principles of this method now following, it has been assumed that a cation Q^+ has to be extracted from an aqueous medium (Q^+ can be a quaternary ammonium compound or a protonated weak base).
A cation Q^+ can be extracted with an organic solvent from an aqueous solution, by adding (at least) an equivalent amount of an anion X^-. These

two ions form an ion pair, which can be transferred to the organic phase, in accordance with the following equation:

$$Q^+_{aq} + X^-_{aq} \rightleftarrows QX_{org} \qquad (I)$$

where:
Q^+_{aq} = the cation, dissolved in the aqueous phase
X^-_{aq} = the anion, dissolved in the aqueous phase
QX_{org} = the ion pair formed, present in the organic phase

The equilibrium constant, which is also called extraction constant, is:

$$E_{QX} = \frac{[QX_{org}]}{[Q^+_{aq}] \times [X^-_{aq}]} \qquad (II)$$

where:
$[Q^+_{aq}]$ = the concentration of the cation in the aqueous phase
$[X^-_{aq}]$ = the concentration of the anion in the aqueous phase
$[QX_{org}]$ = the concentration of the ion pair in the organic phase

Equation (II) is only valid if no side reactions occur. If side reactions do occur, the equation will be written as follows:

$$E^*_{QX} = \frac{c'_{QX_{org}}}{c'_{Q^+_{aq}} \times c'_{X^-_{aq}}} \qquad (III)$$

in which:
E^*_{QX} = the conditional, *i.e.* the actually measured, equilibrium or extraction constant
$c'_{Q^+_{aq}}$ = the concentration of cations Q^+ in the aqueous phase after equilibrium is reached (so, the amount of Q^+, not extracted as ion pair).
$c'_{X^-_{aq}}$ = the concentration of anions X^- in the aqueous phase after equilibrium is reached
$c'_{QX_{org}}$ = the total concentration of the cations, present in the organic phase as ion pairs with X^-.

RINGBOM (82) has shown, that the influence of side reactions in a calculation can be incorporated by using a "conditional extraction constant". The relation between the stoechiometric and the conditional extraction constant is also given by:

$$E^{*}_{QX} = E_{QX} \times \frac{\alpha_{QX}}{\alpha_{Q} \times \alpha_{X}} \qquad \text{(IV)}$$

where the α-coefficients are the quotients of the conditional and the stoechiometric concentrations (*e.g.* $\alpha_{QX} = c'_{QX_{org}}/[QX_{org}]$).

The distribution of Q^+ between the two phases will be given by the distribution coefficient D_{QX}. D_{QX} is the quotient of the amount of Q^+ present in the organic phase as ion pair (QX) and the amount Q^+ that is still present in the aqueous phase after equilibrium has been reached. (The quantity of Q present as ion pair in the aqueous phase is negligible). When the volumes of the aqueous and the organic phase are equal, the following equation will be valid:

$$D_{QX} = \frac{c'_{QX_{org}}}{c'_{Q^+_{aq}}} \qquad \text{(V)}$$

When no side reactions occur, $c'_{QX_{org}} = [QX_{org}]$ and $c'_{Q^+_{aq}} = [Q^+_{aq}]$.

From (II) and (V) follows:

$$D_{QX} = E_{QX} \times [X^-_{aq}] \qquad \text{(VI)}$$

When the conditional extraction constant is known, the percentage of Q^+ extracted in one extraction can be calculated from equation (VII)

$$P = \frac{100}{1 + \frac{V_{aq}}{V_{org} \times D_{QX}}} \qquad \text{(VII)}$$

where (see 91):

P = the percentage of Q^+ obtained in one extraction (expressed as the percentage of the original amount in the aqueous phase)

V_{aq} = the volume of the aqueous phase

V_{org} = the volume of the organic phase

1.2. Parameters influencing the extraction

In ion pair extraction we are dealing with an equilibrium. Various factors determine this equilibrium *e.g.*:
a. the concentration of the different ions, especially $[X^-_{aq}]$
b. the properties of the ions
c. the properties of the ion pair
d. the properties of the extraction solvent

Ad a. *The concentration of the different ions, especially* $[X^-_{aq}]$
The relation between the extraction constant and the concentration of the counter ion $[X^-_{aq}]$ is given by equation (VI). As in general it is desirable that in a single extraction at least 99% of Q^+ is transferred to the organic phase, it is necessary that $D_{QX} \geqslant 100$.
After the extraction constant for a special ion pair extraction has been found, it can be calculated how much of the counter ion X^- should be added to ensure that $D_{QX} \geqslant 100$. The concentration of the ion that is to be extracted is also important, because by this concentration it will be decided whether any side reactions (*e.g.* association in the aqueous phase) will occur that may influence the conditional extraction constant (see below).

Ad b. *The properties of the ions*
The separate ions should easily dissolve in water. If possible, one of the ions should be of an organic nature. The organic molecule should not contain any other strongly polar group except the ionized group (*i.e.* it should be sufficiently lipophilic). The ion added and the ion to be extracted should form a water insoluble compound; in other words, the ion pair formed should not, or very little, be dissociated in water. The pH of the aqueous phase is of course important if one of the ions (or both) is a protolyte (86). The influence of the anion on the extraction constant is shown in Table 1. The table gives the logarithm of the various extraction constants for the quaternary tetrabutylammonium ion as ion pair with several anions. The organic phase is chloroform. Changing of the anion can cause a change of the log E value of several log-units! As one can see, the extraction constant increases in the series of inorganic anions: $Cl^- < Br^- < I^- < ClO_4^-$. This is probably due to decreased hydratation of the anion in the aqueous phase. Apart from the inorganic anions of Table 1, ion pair extractions are also reported with $H_2PO_4^-$, HSO_4^-, SO_4^{2-} and HCO_3^- as a counter ion (100).

Ad c. *The properties of the ion pair*
The properties of the ion pair are closely related to those of the separate ions as discussed under b. The influence of the structure of the ion pair on the

TABLE I: *The various extraction constants of ion pairs between tetrabutylammoniun ion and various anions (organic phase:* $CHCl_3$*) (See reference 67).*

Class	Anion (X^-)	log E_{QX}
Inorganic ions	Hydroxyde	−1.67
	Chloride	−0.11
	Bromide	1.29
	Nitrate	1.39
	Iodide	3.01
	Perchlorate	3.48
Phenols	Phenolate	−0.03
Carboxylic acids	Acetate	−2.12
	Phenylacetate	0.27
	Benzoate	0.39
	Salicylate	2.42
Aromatic sulphonic acids	Toluene-4-sulphonate	2.33
	Naphtalene-2-sulphonate	3.45
Aromatic nitro compounds	Trinitrobenzenesulphonate	4.47
	Trinitrophenolate (= picrate)	5.91
	Hexanitrodiphenylamine (= dipicrylamine)	9.6+
Indicators	Bromothymol blue	8.0+
	Methyl orange	5.47

+ = estimated values

size of the extraction constant can be seen from the following example (70).

Imipramine (a tertiary amine) can easily form an ion pair with chloride in acid medium. This ion pair can be extracted with chloroform: log E = 2.14. If one of the methyl groups of the nitrogen atom is removed, the secondary amine desipramine is formed. The corresponding log E value for desipramine is 0.34, *i.e.* the extraction of imipramine chloride with chloroform is 40–50 times better than the extraction of desipramine chloride!

Moreover, the structure of the counter ion has an important influence on the hydrophilicity-lipophilicity balance of the ion pair and for this reason it can determine the solubility of an ion pair in a certain solvent.

Furthermore occurrence of any side reactions is of importance. In the aqueous phase association of ion pairs is the most important side reaction. Some ion pairs will easily polymerise in the organic phase, whereas others just dissociate in this phase. The occurrence of side reactions in the organic phase will - apart from the properties of the ion pair - also be determined by the properties of the extraction solvent, as showed below.

Ad d. *The properties of the extraction solvent*

The properties of the extraction solvent used are extremely important. The possibility to dissolve the ion pair in it has a great influence on the extraction constant. The properties of the solute (= the ion pair) and of the solvent and especially the polarity and the tendency of these two to form hydrogen bonds which each other, determine the degree of solvation of an ion pair in an organic solvent.

Most ion pairs have a weakly polar character, which causes chlorinated hydrocarbons as chloroform, dichloromethane and 1,2-dichloroethane to be generally useful extraction solvents. These solvents happen to have a weakly polar character - like dissolves like - and have a rather strong tendency to form hydrogen bonds. Within certain limits the extraction constants increase with the polarity of the organic phase. Some examples are given in Table 2, which gives the dielectric constant of some solvents and also the various extraction constants of the picrate ion pair of tetrabutylammonium in these solvents.

TABLE 2: *The various extraction constants for the picrate ion pair of tetrabutylammonium ion (data from reference 35; dielectric constants from reference 42).*

Organic phase	Dielectric constant of the organic phase	log E_{QX}
carbon tetrachloride	2.24	1.94
benzene	2.28	3.59
chloroform	4.8	5.91
dichloromethane	8.9	6.68

During our efforts to establish the best conditions for the ion pair extraction of thiazinamium methylsulphate (see also Chapter 2 of this Part), we found an increasing extraction of thiazinamium as perchlorate ion pair in the series chloroform-dichloromethane-1,2-dichloroethane (Table 3).

TABLE 3: *The various extraction constants for the perchlorate ion pair of thiazinamium ion (see also Chapter 2; dielectric constants from reference 42).*

Organic phase	Dielectric constant of the organic phase	log E_{QX}
chloroform	4.8	2.31
dichloromethane	8.9	3.51
1,2-dichloroethane	10.6	3.71

Generally alcohols with an chain length of C_4-C_7 are also good extraction solvents for ion pairs due to their polarity and because they are both good proton donors and proton acceptors. Especially 1-butanol, 1-pentanol and 1-hexanol are commonly employed.
The properties of the solvent can be altered as the need arises by taking mixtures of them, *e.g.* a mixture of chloroform and 1-pentanol, if necessary mixed with a less polar solvent like cyclohexane, until the exact degree of polarity is obtained.

The extraction constant is ultimately determined by the sum of the factors mentioned under a to d. The occurrence of side reactions plays an important role. Generally speaking, association of the ion pair to polymers is likely to take place in the aqueous phase if the concentrations of the ion to be extracted and of the counter ion are rather high (*i.e.* concentrations of about 10^{-3} mol/l). This causes a decrease of the extraction constant (85, 89). Dissociation of the ion pair in the organic phase, however, is a frequently occurring side reaction, especially in the case of low concentrations (in the order of 10^{-6} mol/l). This phenomenon increases the extraction constant. If dissociation of the ion pair in the organic phase is the only side reaction, the conditional extraction constant can be calculated from the following equation (see BORG: 8):

$$E^*_{QX} = E_{QX} + (E_{QX} \times K_{diss})^{\frac{1}{2}} \times ([Q^+_{aq}] \times c'_{X^-_{aq}})^{-\frac{1}{2}} \qquad \text{(VIII)}$$

where K_{diss} = the dissociation constant of the ion pair QX in the organic phase.
One can see from equation VIII that the conditional extraction constant increases with decreasing values of $([Q^+_{aq}] \times c'_{X^-_{aq}})$, so the extraction of low quantities is favoured by this dissociation in the organic phase. The properties of the organic phase determine if any dissociation will take place. In the course of our own investigations, we found that the ion pair thiazinamium iodide does not dissociate in chloroform, that it slightly dissociates in dichloromethane; but it dissociates to a fair degree during extraction with 1,2-dichloroethane in which $K_{diss} = 7.3 \times 10^{-5}$ (see also Chapter 2 of this Part). This dissociation causes the log E^* value to increase from 2.5 for dichloromethane to 3.1 for 1,2-dichloroethane.

1.3. Quantitative determination of the ion pair

Having been isolated, the ion pair should be determined in a quantitative way. The most commonly used methods for quantitative determination of ion pairs are titrimetric analysis, spectrophotometry, spectrofluorimetry and gas chromatography.

Titrimetric analysis is mentioned here only for the sake of completeness and to indicate that the method (amphimetry) is based on ion pair formation (62, 99, 45). Because this method can only be used for measuring large concentrations (milligram range) it cannot be used in bioanalysis and does not fit in with this Chapter (A few more details and references to literature are given in Part I, Section 4.7.2.).

Spectrophotometry is the most commonly applied method and has been in use for several years after BRODIE and UDENFRIEND introduced this technique for the determination of drugs in plasma by means of ion pair extraction (15, 16). A great advantage of this method is its universality. Absorption of both ultraviolet and visible light can be measured. Ions (*e.g.* drugs) that possess a characteristic U.V.-spectrum of their own (with sufficient molecular extinction) can best be extracted with a counter ion without U.V.-absorbing properties (*e.g.* phenothiazine derivatives with perchlorate). If the drug does not possess a characteristic spectrum one should use a counter ion with a characteristic spectrum and sufficient molecular extinction. In such cases often indicators from titrimetric analysis are used, because these all have characteristic absorption spectra in the visible light (*e.g.* bromothymol blue, methyl orange). Anions such as picrate and dipicrylamine can be very useful, too.

These ions are generally very suitable for ion pair extraction because they can give high extraction constants (see Table 1). Concentrations down to 1 μg/ml can be measured in this way with acceptable precision and accuracy.

Spectrofluorimetry has two important advantages over spectrophotometry: it allows detection of lower quantities and it can be more specific. With the help of counter ions like anthracene-4-sulphonate, 9,10-dimethoxyanthracene-2-sulphonate or N,N,-dimethylprotriptyline, concentrations down to 10 ng/ml are detectable (54, 108).

A disadvantage of the spectrofluorimetric detection method is, however, the limited choice of counter ion. Furthermore, with this method disturbances may occur. The buffer solutions and extraction solvents should be of an extremely high degree of purity to avoid blank values.

Both methods can directly be applied if there are no interfering compounds present. In the cases of complex mixtures, which most body fluids are, it will usually be necessary to carry out a purification step between extraction and detection. This clean up procedure can take place with the aid of thin-layer

chromatography, column chromatography (*e.g.* high pressure liquid chromatography) or by pre-extraction or re-extraction (see 1.5).
In the past few years there has been an increasing interest in the spectrophotometric detection method. This increase is caused by the fact that it can easily be combined with a separation by means of liquid chromatography (22, 26, 30, 50, 76, 97).
The modern liquid chromatographs are usually equipped with good spectrophotometric and spectrofluorimetric detectors. For this reason it is possible to obtain purified extracts and to detect very low quantities in them.
When a *gas chromatographic* method is utilized, separation and detection also take place directly after each other. In literature there are only a small number of reports about the combination of ion pair extraction and gas chromatography (7, 48, 49, 102, 103, 104). This is explained by the generally low volatility of ion pairs.
Application of gas chromatography is only possible when the ion pair can easily be converted into a more volatile compound (*e.g.* by pyrolysis). An advantage of gas chromatography is the large sensitivity, especially when using selective detectors as alkali flame ionization detectors and electron capture detectors and, in some cases, flame photometer detectors. With such apparatus concentrations of appr. 1 ng/ml are detectable.

1.4. Different methods in the ion pair extraction technique

1.4.1. Solvent-solvent extraction ("batch extraction technique")

When ion pair extraction of a drug from biological materials is required, one must begin by trying to find a counter ion and an extraction solvent that will give a sufficiently high conditional extraction constant. If at least one of the components of the ion pair is a protolyte, determination of the pK_a and the distribution coefficient of this component should precede determination of the conditional extraction constant.
From the values obtained data can be inferred as to the optimum circumstances (especially the pH-value). An example of the influence of the pH on separation is given by SCHILL (91). Both atropine and pralidoxime are extractable as ion pair with bromothymol blue. The most important side

reactions are protolysis and partition of atropine as a base and of bromothymol blue as an acid. Bromothymol blue cannot be employed as an extraction reagent below a pH value of 7.5, otherwise the unionized acid will also be extracted, which would render spectrophotometric detection impossible. A quantitative separation between atropine and pralidoxime appears to be possible between pH = 8.4 and pH = 8.8.

The selection of experimental conditions is mainly determined by the magnitude of the conditional extraction constant. In the first instance this can be roughly estimated for several systems with different counter ions and different extraction solvents (for the choice of the counter ion and the extraction solvent the data mentioned in literature for components with approximately the same molecular structure may be helpful). In such a preliminary determination one can use *e.g.* an initial concentration $[X^-_{aq}] = [Q^+_{aq}]/2$. In that case no complete extraction is achieved, and concentrations can be measured in both phases with satisfactory precision. The ions are preferably to be dissolved in buffer solutions. (This is not strictly necessary when dealing with quaternary ammonium compounds, but the exact pH is of high importance when X, or Q, is a weak base or a weak acid).

The solution of Q^+ is brought into a centrifuge tube with Quickfit® stopper and an identical volume of a solution of X^- is added. Next, so much of the extraction solvent is added, that both the organic and the aqueous phase have the same volume. Then the two phases are shaken in a water bath of constant temperature until equilibrium has been reached. In most cases centrifugation is necessary to separate the phases. In both phases the concentration of Q is then measured (in the aqueous phase as Q^+ and in the organic phase as ion pair QX). By means of equation (V) the distribution coefficient D_{QX} is calculated. With this value of D_{QX} the extraction constant can then be calculated from equation (VI) (67).

It has been pointed out (SCHILL, personal communication, 1974), that disturbing side effects can be avoided to a great extent when the ionic strength < 0.1, which means $[X^-_{aq}] < 0.1$. An almost complete extraction ($D_{QX} \geqslant 100$) can therefore only be obtained with extraction constants $\geqslant 10^3$ ($\log E_{QX} \geqslant 3$). If, for the system under investigation, one actually finds an E_{QX}-value of approximately 10^3, the preliminary estimation can be followed by an exact determination, now with several concentrations of X^-. If no significantly different values are found for E_{QX}, this means that concentration-dependent side reactions are absent. More details about investigation of side reactions are given in (91).

After the extraction constant has been determined for the extraction from water (or a buffer solution) it can be calculated how much of the counter ion X^- is required to get a quantitative extraction of therapeutic concentrations of drugs.

However, it is impossible to use this extraction constant for an extraction

from biological fluids, such as plasma because it may happen that certain plasma components also react with the counter ion X^- (*e.g.* binding to plasma proteins). For this reason it is necessary to determine the conditional extraction constant for extraction from spiked plasma as well; if required, the concentration of counter ions should be increased (108).

1.4.2. Extraction by means of ion pair partition chromatography

Beside the batch extraction technique isolation by means of ion pair partition chromatography has been extensively used in the past few years. The specific properties of an ion pair extraction system can be utilized successfully in liquid chromatography if the amount and the composition of the stationary phase are known and the influence of the support on the migration velocity of the sample is negligible. High concentrations of stationary phase on inert porous supports (*e.g.* diatomaceous earth, cellulose), are frequently used for ion pair partition chromatography.
EKSBORG and SCHILL (30) published the results of a comprehensive study concerning ion pair partition chromatography ("straight phase") of quaternary alkylammonium ions. Cellulose was used as support, an aqueous solution of picrate as stationary phase and a chloroform/1-pentanol mixture (19 : 1) as mobile phase. Apart from this mainly theoretical study several applications for drug analysis have been described (11, 12, 22, 27, 28, 61, 71, 74, 80). In most of these publications chloroform or dichloromethane has been employed as mobile phase.
The use of mixtures of solvents has also been reported in particular combinations with 1-pentanol. Aqueous solutions of picrate, dipicrylamine or perchlorate are often used as stationary phase. Cellulose or Celite (= diatomaceous earth) are mentioned as support.
Besides, "reversed phase chromatography" is often applied, both as thin-layer chromatography and as column chromatography. In most cases silicone-treated cellulose or acetylated cellulose is applied as support (MN 2100 W.A. and MN 2100 AC, respectively; Machery, Nagel & Co., Düren, G.F.R.). There, lipophilic alcohols, *e.g.* 1-butanol, 1-pentanol or 1-hexanol, are used as stationary phase. As mobile phase only aqueous solutions of inorganic anions have been reported (*e.g.* Cl^-, Br^-, ClO_4^- and SO_4^{2-}). The method of "reversed phase chromatography" has been described in particular for some quaternary ammonium compounds and a number of drugs from the group of phenothiazine derivatives and tricyclic antidepressive drugs (imino-dibenzyl derivatives). The "reversed phase" technique offers some practical advantages over the "straight phase" methods. In a "reversed phase" system the counter ion has been dissolved in the mobile phase. This enables easy changing of the nature or the

concentration of the counter ion, if this should be necessary *e.g.* in cases of bad separation. Another advantage is the possibility to apply the quaternary ammonium compound to the system, dissolved in a small volume of water (33, 34, 105). No applications of the "reversed phase" system in the bioanalysis have been reported.
A new development in this area is the combination of ion pair partition with high performance liquid-liquid chromatography (H.P.L.C.), as recently described by PERSSON and KARGER (76). This method was used by them in the separation of several biogenic amines and their metabolites in aqueous solutions. For the separation of the amines and amino acids organic solvents such as butanol, ethylacetate mixed with dichloromethane or hexane have been used as mobile phases and a mixture of perchloric acid and sodium perchlorate as a stationary phase, coated on porous silica particles (10 μm diameter). For the separation of the metabolites (carboxylic acids) tetrabutylammonium ion has been used as counter ion in buffer solutions of carefully selected pH-ranges.
The same group (50) described a different application of this method, based on the combination of high performance liquid-liquid chromatography and highly selective ion pair formation, namely the separation of thyroid hormones and the separation of twelve sulphonamides, both in aqueous solutions. For the thyroid hormones the stationary phase was a mixture of perchloric acid and sodium perchlorate (on silica gel), and the mobile phase was a butanol/dichloromethane mixture (15:85). For the sulphonamides the stationary phase was tetrabutylammonium sulphate and borate buffer (pH = 9.2) on silica gel, with a butanol/hexane mixture (25:75) as a mobile phase. In a later paper (97) the results of a study for optimalization of the physico-chemical parameters for the system were described.

1.5. Applications and possibilities

General

Many publications on ion pair extraction from water have appeared. Especially in the past few years an increasing number of publications have appeared about isolation of drugs and drug metabolites in several body fluids.
In most cases the procedures for the isolation from water can be taken as a basis for isolation from blood or urine, but in general an extra clean up

procedure is required. Several possibilities are available to obtain a clean extract.

In the first place a *pre-extraction* can be carried out. To this end the plasma (or urine) is extracted with the organic solvent, which is used for the ion pair extraction, but before adding the counter ion. All plasma components soluble in this solvent are removed from the plasma in this way and will not pollute the ion pair extract. Then the counter ion is added and the ion pair extraction can take place.

A *re-extraction* can also be carried out, in particular to remove non-ionized partly water-soluble impurities. In this method the counter ion is directly added and the ion pair is extracted with the organic solvent (*e.g.* from plasma). The phases are separated and an aliquot of the organic phase is transferred to another extraction tube. Next, an aqueous solution of the counter ion is added to this organic liquid which is then shaken again. The ion pair of the drug will stay in the organic solvent (mainly by the excess of counter ions). The interfering plasma components, however, are largely extracted back to the aqueous phase, because they do not form ion pairs with the counter ion and also on account of their being partly soluble in the organic phase by themselves. An application of the re-extraction technique is found in a publication of BORG and associates (10), in which they describe the determination of Piribenzyl® (= bevonium methylsulphate) in urine as ion pair with bromothymol blue. Beside this drug a metabolite and a few other interfering components (*e.g.* choline, nicotine, tyramine, tryptamine) are present in the urine. The disturbing components, however, have lower extraction constants for the extraction with dichloromethane than Piribenzyl®, but they are present in far higher concentrations, so they may cause considerable disturbance. Without re-extraction the blank extinction was 1.1 and after two re-extractions it was only 0.1. The recovery for Piribenzyl® was 97%.

It is by no means imperative that the counter ion of the first extraction should be the same for the re-extraction. This has been shown for example in a publication of EKSBORG and others (29), who describe the extraction procedure for the quaternary ammonium compound emepronium bromide (= Cetiprin®) in urine. First emepronium is extracted from urine with perchlorate as counter ion and dichloromethane as extraction solvent. The organic phase is re-extracted with a solution of perchlorate in a phosphate buffer of pH = 12. After this a second re-extraction follows, now with bromothymol blue as counter ion. The second re-extraction is possible because the ion pair of emepronium with bromothymol blue has a higher stability constant than the ion pair with perchlorate. The ion pair of emepronium with bromothymol blue has a high molecular extinction at 635 nm and can be measured spectrophotometrically.

A third procedure to clean up the extract is the use of *chromatography* (liquid or gas chromatography). The extract is concentrated, after which an

aliquot is submitted to a chromatographic process. When liquid chromatography is used, an aqueous solution of the counter ion is the stationary phase (in a "reversed phase" technique, however, it is the mobile phase). EKSBORG and PERSSON (28) described a method for the analysis of the quaternary ammonium compound choline in a biological material (urine) using liquid chromatography. Choline is extracted from urine as ion pair with dipicrylamine in dichloromethane. The dichloromethane extract is concentrated and picrate (as tetrabutylammonium picrate) is added. The mixture is quantitatively injected on the separation column of the liquid chromatograph. The stationary phase is sodium picrate and a dichloromethane/1-pentanol mixture (98:2) is the mobile phase. So choline is eluted as picrate and the concentration is spectrophotometrically determined. Measurements in the microgram range are possible.

BORG (9) developed a method for the quantitative analysis of pentazocine in plasma. Pentazocine is extracted as such from plasma by means of benzene. Next, the extract is purified by means of partition chromatography. Pentazocine is transferred to the column as ion pair with chloride; 0.1 molar hydrochloric acid is used as stationary phase and a cyclohexane/1-pentanol mixture (60 : 40) as mobile phase. The support is cellulose. In the eluate pentazocine is determined spectrophotometrically.

A gas chromatographic procedure can also be used for further purification of the extract. When this method is applied, the selectivity and the sensitivity can be increased by the application of selective detectors.

As a matter of course, application of gas chromatography is only possible if the sample can be easily transferred into the gaseous phase. If this is not the case, the sample (*e.g.* drug or metabolite) should be subjected to pyrolysis or derivatization, BOON and MACE (7) described a gas chromatographic determination of some drugs (*e.g.* tripelennamine, chlorpromazine and pethidine) as ion pair. Ion pairs are formed with bromothymol blue as a counter ion and extracted by means of chloroform. The ion pairs are split into the respective amines in the gas chromatograph. These amines are volatile and can be gas chromatographically determined. VIDIC and others (104) also described splitting of the ion pair. These authors extracted quaternary ammonium compounds as ion pair with iodide. The ion pair is split in a special pyrolysis oven (800° C) and the alkyl iodide formed (methyl, ethyl or butyl iodide) is detected by means of a flame ionization detector (detection limit appr. 1 μg). However, the low temperature of the oven of the gas chromatograph (50°–90° C) can cause contamination of the column. A second disadvantage of the method is its lack of specificity. From our own investigations (48, 49, see also Part II, Chapter 2) it has become apparent that pyrolysis of the ion pair thiazinamium iodide already occurs in the injection part of the gas chromatograph (at 300° C), which causes the ion pair to split into methyl iodide and the tertiary amine, promethazine. The latter is volatile and is detected by means of an alkali flame ionization

detector (detection limit 2 ng/ml plasma). At the relatively high oven temperature (240° C) both pyrolysis products are volatile and no column contamination occurs.

BERLIN and others (5) and VESSMAN and others (103) also used a combination of ion pair extraction and gas chromatography but they did not inject the ion pair as such into the gas chromatograph. BERLIN extracted bupivacaine as an ion pair with perchlorate and dichloromethane as solvent. After that, the ion pair was split and the base injected into the gas chromatograph. VESSMAN extracted the quaternary ammonium compound emepronium in the same way. Next, the ion pair was oxidized to the corresponding benzophenone which was detected by means of an electron capture detector.

It is also possible to make a volatile derivative of the drug (or its metabolite) and determine this derivative by means of a gas chromatograph. A new development in this field is the application of an "extractive alkylation technique" for analysis of drugs in biological materials (24, 25, 31). This method is based on the high reactivity of some anions in nucleophilic substitution reactions in weakly polar solvents. The process of "extractive alkylation" takes place when an anion is extracted as ion pair with a quaternary ammonium compound and with the aid of an organic solvent (*e.g.* dichloromethane) containing an alkylating reagent (*e.g.* an alkyl halide). The alkylation takes place very rapidly and the products formed are sufficiently volatile for a gas chromatographic procedure to be applied. ERVIK and GUSTAVII (31) describe the quantitative determination of chlorthalidone in the nanogram range in plasma, using an "extractive alkylation technique" followed by gas chromatography with electron capture detection. Because three polar active groups are present in the chlorthalidone molecule, direct gas chromatography of the drug as such is impossible. The active groups contain four hydrogen atoms, bound to hetero atoms. Chlorthalidone is extracted in an alkaline medium from plasma with methyl-isobutylketone. The solvent is then evaporated, after which a solution of tetrahexylammonium hydrogensulphate is added to the residue. The quaternary ammonium compound forms an ion pair with chlorthalidone. This ion pair is extracted with dichloromethane in which the alkylating reagent alkyl iodide is dissolved. Next the dichloromethane is evaporated. The residue (*i.e.* the tetramethyl derivative of chlorthalidone) is dissolved in hexane. The derivative is far more volatile than the original chlorthalidone, which enables determination by means of gas chromatography. The detection limit is 2 ng/ml plasma.

EHRSSON and TILLY (25) apply the "extractive alkylation technique" for gas chromatographic determination of nitrazepam in plasma. The drug is converted to a methyl derivative. The detection limit is 5 ng/ml plasma.

On the whole the possibilities of the "extractive alkylation technique" are great; there is little doubt that in the near future it will be applied for the

determination of a significant number of drugs in body fluids.
In some cases purification of the extract by means of re-extraction or by a chromatographic process is not enough. Especially when determining very low concentrations of a drug, the extract must be very clean indeed.
For that reason it may at times be necessary to combine re-extraction with, for instance, column chromatographic separation. An example of such a combination is given by EKSBORG and PERSSON (27) for the determination of acetylcholine in rat brains. Acetylcholine, a quaternary ammonium compound, is extracted as ion pair with dipicrylamine from homogenized brain tissue. The extraction solvent is dichloromethane. After extraction the dichloromethane is evaporated. A solution of picrate in water is added to the residue and the mixture is transferred quantitatively to a separation column (support: cellulose). The picrate solution functions as stationary phase and a chloroform/pentanol mixture is used as a mobile phase. Acetylcholine migrates as ion pair with picrate over the column. The eluate is considerably cleaner than the original extract of the dipicrylamine ion pair. However, for reliable quantitation it is necessary to re-extract the extract with an alkaline aqueous phase. During re-extraction choline is formed by hydrolysis, after which the choline is extracted as ion pair, with dipicrylamine as counter ion and dichloromethane as solvent. Detection is performed spectrophotometrically (detection limit is 1 μg/ml).

N.B. During the final stage of preparing this part of the manuscript ULIN and others reported a method for the bioanalysis of picomole amounts of acetylcholine in rat sciatic nerve. This method is based on the same principles as the method described above, but the sensitivity was increased by about 50 times by selecting another extraction agent (3,5-di-t-butyl-2-hydrobenzene sulphonate) and adapting modern liquid chromatography with high-sensitive on-line ultraviolet detection. (B. ULIN, K. GUSTAVII, and B. A. PERSSON, 1976, J. Pharm. Pharmacol. 28, 672).

1.6. Some examples of the application of ion pair extraction in drug analysis

A number of applications of the ion pair extraction technique is shown in Tables 4 to 8. In these tables several publications are mentioned, in which the analysis of drugs according to this method is described. Apart from methods describing drug analysis in body fluids, methods for the quantitative analysis of drugs in aqueous solutions (or dosage forms) are also mentioned.
This has been done because the latter methods may be adapted to analysis in body fluids, for instance after using a clean up procedure (as described in 1.5.).
Ion pair extraction is the first method to be selected for isolating compounds from aqueous media that are completely ionized at all pH values such as quaternary ammonium compounds, amphoteric sulphonic acids and amphoteric carboxylic acids (*e.g.* amino acids). Primary, secondary and tertiary amines can also be extracted as ion pairs, but only if these compounds are in a protonated form, hence only in an acid medium. Ion pair extraction in an acid medium has the advantage of higher selectivity over extraction as a base in an alkaline medium. It is a prerequisite of course, that the drug should not decompose in the acid medium. The selectivity of the ion pair extraction can further be increased by selecting optimal pH, counter ion and extraction solvent as mentioned before (23).
The drugs will be dealt with in groups. Only drugs known in the Netherlands come up for discussion.
In the tables the following abbreviations are used:

G.L.C. = gas chromatography
H.P.L.C. = high pressure liquid chromatography
S.F. = spectrofluorimetry
S.P. = spectrophotometry
T.L.C. = thin-layer chromatography

The drugs are indicated by the name of the bases.

1.6.1. Primary amines

It is for only a small number of primary amines, that ion pair extraction methods have been described in literature. In most cases detection is performed by means of spectrophotometry. In Table 4 the data about ion pair extraction of primary amines are summarized.

TABLE 4: *Ion pair extraction of primary amines*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
Amphetamine	1	toluene-4-sulphonic acid	$CHCl_3$	water	S.P.	22
	2	bis(2-ethylhexyl) phosphoric acid	$CHCl_3$	water	S.P.	65
	3	bromothymol blue	CH_2Cl_2	water	S.P.	87
Chlorthalidone		-	"extractive alkylation"	plasma	G.L.C.	31
Dopamine	1	-	column partition chromatography Support: cellulose Stat. phase: Cl^-, ClO_4^- Mob. Phase: 1-pentanol + cyclohexane	water	S.P.	74
	2	-	H.P.L.C.: (partition chromatography) Support: silica Stat. phase: ClO_4^- Mob. phase: butanol, ethylacetate + CH_2Cl_2 (or hexane)	water	S.P.	76
Mescaline		bromocresol blue	$CHCl_3$	plasma urine	S.P.	109
Noradrenaline	1	as amphetamine-2				65
	2	as dopamine-2				76
Tryptamine	1	as dopamine-1				74
	2	as dopamine-2				76

1.6.2. Secondary amines

As concerns the group of secondary amines most attention has been paid to ion pair extraction methods for a number of sympathomimetic drugs and some tricyclic antidepressive drugs. Detection generally takes place by spectrophotometry or, *e.g.* with the sympathomimetic drugs, by spectrofluorimetry.

The data about ion pair extraction of secondary amines have been summarized in Table 5.

TABLE 5: *Ion pair extraction of secondary amines*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
Adrenaline	1	bis(2-ethylhexyl)-phosphoric acid	$CHCl_3$	water	S.P.	65
	2	as dopamine-2 (Table 1)				76
Bupivacaine		ClO_4^-	CH_2Cl_2	plasma	G.L.C.	5
Desipramine	1	–	thin-layer chromatography ("reversed phase") Support: acetylated cellulose Stat. phase: 1-butanol or 1-pentanol Mob. phase: Cl^-, Br^- or ClO_4^-	water	S.P.	33
	2	Cl^-	$CHCl_3$	water	S.P.	70
	3	Cl^-	1) cyclohexane + lipophilic alcohols 2) column partition chromatography Support: cellulose Stat. phase: Cl^- Mob. phase: cyclohexane + lipophilic alcohols	water	S.P.	71
	4	Cl^-, Br^-, ClO_4^- naphtalene-2-sulphonic acid bromothymol blue	$CHCl_3$, CH_2Cl_2 or $C_2H_4Cl_2$	water	S.P.	75
	5		column partition chromatography ("reversed phase") Support: siliconated cellulose Stat. phase: 1-pentanol, 1-hexanol Mob. phase: Cl^-, SO_4^{2-}	water	S.P.	105
	6	anthracene-2-sulphonic acid	CH_2Cl_2	water	S.F.	107
Ephedrine	1	bromothymol blue	$CHCl_3$	water	S.P.	32
	2	as adrenaline				65
	3	as amphetamine-3 (Table 4)				87
Isoprenaline		as adrenaline				65
Naphazoline		as desipramine-5				105
Nitrazepam		–	"extractive alkylation"	plasma	G.L.C.	25
Nortriptyline	1	as desipramine-2				70
	2	as desipramine-5				105
	3	as desipramine-6				107
Phenylephrine		as amphetamine-3 (Table 4)				87
Protriptyline	1	as desipramine-2				70
	2	as desipramine-5				105
	3	as desipramine-6				107
	4	Cl^-, ClO_4^-, $H_2PO_4^-$	$CHCl_3$ + 1-pentanol	water	S.P.	73
	5	methyl orange	$CHCl_3$, $C_2H_4Cl_2$	water	S.P.	69
	6	Cl^-, ClO_4^-, $H_2PO_4^-$	$CHCl_3$, $C_2H_4Cl_2$	water	S.F.	72
	7	anthracene-2-sulphonic acid	CH_2Cl_2	water	S.F.	107
Synephrine	1	as adrenaline				65
	2	as amphetamine-3 (Table 4)				87

1.6.3. Tertiary amines

As regards the tertiary amines, the ion pair extraction method has been comprehensively studied for several tricyclic antidepressive, antihistaminic and neuroleptic drugs and alkaloids. The extraction procedures described for the tertiary amines from the group of tricyclic antidepressive drugs (cycloheptadiene or azepine derivatives) are in many cases similar to those for the secondary amines from the same group (see 1.6.2.). The same methods can also be used for isolating of phenothiazine derivatives with neuroleptic and/or antihistaminic action. Many methods have been described for the isolation of alkaloids and antihistaminic drugs.
The ion pair extraction methods for drugs containing a tertiary amine group are summarized in Table 6.

TABLE 6: *Ion pair extraction of tertiary amines*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
Amitriptyline	1	as desipramine-2 (Table 5)				70
	2	as desipramine-5 (Table 5)				105
	3	as desipramine-6 (Table 5)				107
	4	as protriptyline-7 (Table 5)				106
	5	anthracene-2-sulphonate	CH_2Cl_2	water	S.F.	54
Atropine	1	picrate	CH_2Cl_2	water	S.P.	36
	2	-	column partition chromatography Support: Celite Stat. phase: toluene-4-sulphonic acid Mob. phase: $CHCl_3$	water	S.P.	59
Bromopheniramine		picrate	$CHCl_3$	water	S.P.	43
Chlorimipramine		as desipramine-2 (Table 5)				70
Chloropheniramine	1	-	column partition chromatography Support: Celite Stat. phase: toluene-4-sulphonic acid Mob. phase: $CHCl_3$	water	S.P.	22
	2	Cl^-, Br^-, maleate, picrate, trichloroacetate	$CHCl_3$	water	S.P.	39
	3	-	column partition chromatography Support: Celite Stat. phase: toluene-4-sulphonic acid Mob. phase: $CHCl_3$	water	S.P.	57
	4	methyl orange	$CHCl_3$	water + dosage forms	S.P.	19

TABLE 6: *Ion pair extraction of tertiary amines (cont.)*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
Chloroquine	1	as amphetamine-3 (Table 4)				87
	2	as chlorpromazine-5				88
Chlorpromazine	1	as desipramine-2 (Table 5)				70
	2	as desipramine-3 (Table 5)				71
	3	as amphetamine-3 (Table 4)				87
	4	bromothymol blue	$CHCl_3$	water	G.L.C.	7
	5	bromothymol blue	CH_2Cl_2	water	S.P.	88
Cinchonidine	1	methyl orange	$C_2H_4Cl_2$	plasma, urine	S.P.	15
	2	methyl orange	benzene (+ iso-amylalcohol)	plasma, urine	S.P.	16
Cinchonine		as cinchonidine-2				16
Cocaine		as mescaline (Table 4)				109
Codeine	1	as atropine-1				36
	2	as atropine-2				59
	3	as amphetamine-3 (Table 4)				87
	4	NO_3^-	$CHCl_3$	water	S.P.	22
	5	Cl^-, Br^-, I^-, ClO_4^-, NO_3^-, CNS^-	$CHCl_3$ Also: column partition chromatography Support: Celite 545 Stat. phase: I^- Mob. phase: $CHCl_3$	water	S.P.	94
Dextromethorphan	1	-	column partition chromatography Support: Celite Stat. phase: Cl^- Mob. phase: $CHCl_3$	water	S.P.	23
	2	Cl^-, Br^-, I^-, NO_3^- trichloroacetate, picrate, ethylsulphonate, benzene sulphonate	$CHCl_3$ and 1-pentanol (+ cyclohexane)	water	S.P.	41
	3	Br^-, I^-, ClO_4^-, thrichloroacetate	$CHCl_3$ (+ CCl_4)	water	S.P.	40
Dihydrocodeinone		as amphetamine-3 (Table 4)				87
Diphenylhydramine	1	as codeine-4				22
	2	as ephedrine-1 (Table 5)				32
	3	as chloropheniramine-3				57
	4	as chloropheniramine-4				19
	5	tetrabromophenol-phtaleine-ethylester	$C_2H_4Cl_2$	water	S.P.	101
	6	dipicrylamine	$CHCl_3$	water + dosage forms	S.P.	95

TABLE 6: *Ion pair extraction of tertiary amines (cont.)*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
Fluphenazine		as desipramine-2 (Table 5)				70
Hydroxyzine		as desipramine-2 (Table 5)				70
Imipramine		as desipramine-1, -2, -3, -4, -5, -6 (Table 5)				33, 70, 71, 75, 105, 107
Lidocaine	1	as amphetamine-3 (Table 4)				87
	2	as codeine-5				94
	3	as desipramine-5 (Table 5)				105
Meclizine		Br^-, Cl^-	$CHCl_3$	water	S.P.	77
Methadone	1	as amphetamine-3 (Table 4)				87
	2	Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, CNS^-, SO_4^{2-}	$CHCl_3$	water	S.P.	77
Morphine		as amphetamine-3 (Table 4)				87
Nicotine		bromothymol blue	CH_2Cl_2	water, urine	S.P.	10
Opipramol	1	as desipramine-2 (Table 5)				70
	2	as desipramine-5 (Table 5)				105
Papaverine	1	as desipramine-1 (Table 5)				33
	2	as methadone-2				77
	3	as amphetamine-3 (Table 4)				87
	4	as desipramine-5 (Table 5)				105
Pentazocine			column partition chromatography Support: cellulose Stat. phase: Cl^- Mob. phase: cyclohexane/ 1-pentanol (40 : 60)	plasma	S.F.	11
Perphenazine	1	as desipramine-2 (Table 5)				70
	2	as desipramine-3 (Table 5)				71
Pethidine		as chlorpromazine-4				7
Pilocarpine		as amphetamine-3 (Table 4)				87

TABLE 6: *Ion pair extraction of tertiary amines (cont.)*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
Procaine	1	as atropine-1				36
	2	as amphetamine-3 (Table 4)				87
Promazine	1	as atropine-1				36
	2	as amphetamine-3 (Table 4)				87
	3	as codeine-5				94
	4	as desipramine-5 (Table 5)				105
Promethazine	1	as protriptyline-5 (Table 5)				69
	2	as desipramine-5 (Table 5)				105
	3	as chloropheniramine-3				57
Quinine	1	as amphetamine-3 (Table 4)				87
	2	as protriptyline-7 (Table 5)				106
	3	as atropine-2				59
Reserpine		as amphetamine-3 (Table 4)				87
Strychnine	1	as desipramine-1 (Table 5)				33
	2	as atropine-1				36
	3	as amphetamine-3 (Table 4)				87
	4	as codeine-5				94
	5	as desipramine-5 (Table 5)				105
	6	as atropine-2				70
	7	Cl^-	$CHCl_3$	water	gravimetric analysis	37
Trihexyphenidyl		as chloropheniramine-1				22
Trimipramine	1	as desipramine-2 (Table 5)				70
	2	as desipramine-3 (Table 5)				71
	3	as desipramine-5 (Table 5)				105
Tripelennamine	1	as chlorpromazine-4				7
	2	as chloropheniramine-3				57

1.6.4. Quaternary ammonium compounds

In the early 1940-ies there was an increasing use of quaternary ammonium compounds as antimicrobial agents (for conservation and disinfection) *e.g.* benzalkonium, cetrimid and cetylpyridine (2, 32, 81). This was followed by an increase in the interest in methods of quantitative analysis of these compounds. Drugs containing a quaternary ammonium group are especially used as muscle relaxants, (*e.g.* decamethonium, methylcurarine, pancuronium, suxamethonium and d-tubocurarine) and as parasympatholytic drugs (oxyphenonium, propantheline, pyridostigmine and thiazinamium). CHATTEN and OKAMURA (17) published three methods for the determination of 23 quaternary ammonium compounds by means of ion pair extraction. Application to the determination in several dosage forms was also mentioned.

In the past few years there has been a particular interest in the determination of parasympatholytic drugs in body fluids. This is caused by the fact, that the absorption of these drugs after oral administration is known to be at times irregular and incomplete. The determination of these compounds in body fluids and tissues is also important for toxicological reasons. The quantitative determination of such compounds in, for instance, plasma is difficult, because on the one hand the concentrations are very low (nanogram range) and on the other hand isolation is not easy.

The solubility in aqueous media is very high, which is caused by the presence of the quaternary ammonium group(s). Moreover, this solubility cannot be influenced by changing the pH, as can be done with other amines, hence it is impossible to extract the free base in an alkaline medium with *e.g.* hexane. Such an extraction is only possible after a previous demethylation into the tertiary amine. In the literature a few methods have been published, in which demethylation of quaternary ammonium compounds is described. Best known of these is the Hofmann degradation which has been extensively studied for benzalkonium by JENNINGS and MITCHNER (47, 63). JENDEN and others (46) have described the demethylation of acetylcholine with sodium benzothiolate. However both methods have practical disadvantages. The procedure is time consuming and high temperatures must be used, which entails degradation or oxidation reactions. Determination in plasma is difficult, because coagulation of plasma proteins will occur, which hampers complete extraction of the tertiary compound formed.

It will be clear, that ion pair extraction is the most suitable isolation method for this group of ionized compounds.

Although several methods have been published for the determination of quaternary ammonium compounds in water, the number of methods for determination in biological fluids is limited.

In 1974 STEVENS and MOFFAT (96) published a rapid screening procedure

for some quaternary ammonium ions in body fluids and tissues. Their studies include methylatropine, azamethonium, bretylium, cetrimid, decamethonium, hexamethonium, paraquat, suxamethonium, d-tubocurarine, acetylcholine, choline and pancuronium.
The method described is based on ion pair extraction followed by thin-layer chromatography. The detection was carried out by means of spray reagents and the coloured spots were visually compared with a standard. Minimum detectable amount is about 5 µg/ml. The method is unsuitable in case exact information is required (*e.g.* in pharmacokinetic studies) but it may be useful for semi-quantitative work (*e.g.* a toxicological screening).
From this publication and also from other ones, it can be learned, that spectrophotometric detection methods are mostly not sensitive enough for quantitative measurement of low therapeutic plasma concentrations of parasympatholytic drugs. At times even a spectrofluorimetric method will not be sensitive enough to detect therapeutic plasma concentrations. Then, gas chromatography may be a good way to solve this problem (see also 1.5.).
Ion pair analysis of some quaternary ammonium compounds is summarized in Table 7.

TABLE 7: *Ion pair extraction of quaternary ammonium compounds*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
Acetylcholine	1	dipicrylamine	CH_2Cl_2 then: column partition chromatography	brain tissue	S.F.	27
	2	dipicrylamine	CH_2Cl_2	brain tissue	G.L.C.	51
Benzalkonium		bromothymol blue	$CHCl_3$	water	S.P.	32
Butylscopolamine		I_2/I^-	$CHCl_3$	various biol. mat.	G.L.C.	104
Carbachol		as amphetamine-3 (Table 4)				87
Cetrimide		as ephedrine-1 (Table 5)				32
Cetylpyridine	1	I^-	$CHCl_3$	water	S.P.	81
	2	methyl orange	CH_2Cl_2	water	S.P.	17
Choline	1	I_2/I^-	$C_2H_4Cl_2$	plasma	S.P.	1
	2	as acetylcholine-2				27
	3	picrate	$CHCl_3$, CH_2Cl_2, ethylacetate, methylisobutylketone, 1-pentanol	water	S.P.	64
	4	dipicrylamine	CH_2Cl_2	water	S.P.	84

TABLE 7: *Ion pair extraction of quaternary ammonium compounds (cont.)*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
	5	as amphetamine-3 (Table 4)				87
	6	bromothymol blue	CH_2Cl_2, followed by T.L.C.: Support: cellulose Mob. phase: tetrahydrofurane or: Support: silica Mob. phase: methanol	tissues	visual (iodo-platinate formate)	96
	7	as butylscopolamine				104
Decamethonium	1	bromothymol blue	$CHCl_3$ or $C_2H_2Cl_4$	water	S.P.	4
	2	as ephedrine-1 (Table 5)				32
	3	as amphetamine-3 (Table 4)				87
	4	as choline-6				96
Emepronium	1	extraction with ClO_4^-; determination with bromothymol blue	CH_2Cl_2	urine	S.P.	29
	2	-	column partition chromatography ("reversed phase") Support: siliconated cellulose Stat. phase: 1-pentanol Mob. phase: Cl^-, Br^-	water	S.P.	34
	3	as protriptyline-5 (Table 5)				69
	4	as choline-4				84
	5	as amphetamine-3 (Table 4)				87
	6	ClO_4^-, then oxidation	CH_2Cl_2	plasma	G.L.C.	103
	7	as desipramine-5 (Table 5)				105
Hexamethonium	1	as decamethonium-1				4
	2	as ephedrine-1 (Table 5)				32
	3	as choline-4				84
	4	as amphetamine-3 (Table 4)				87
	5	as choline-6				96
Isopropamide	1	methyl orange	$CHCl_3$	water	S.P.	83
	2	methyl orange	CH_2Cl_2	water	S.P.	17
Methylatropine	1	as choline-4				84
	2	as amphetamine-3 (Table 4)				87
	3	as choline-6				96
Methylcurarine		as butylscopolamine				104
Methylscopolamine	1	as choline-4				84
	2	as amphetamine-3 (Table 4)				87

TABLE 7: *Ion pair extraction of quaternary ammonium compounds (cont.)*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
Neostigmine	1	as choline-4				84
	2	as diphenylhydramine-4 (Table 6)				101
	3	as butylscopolamine				104
Oxyphenonium	1	tropaeoline oo	$CHCl_3$	water	S.P.	6
	2	bromophenol blue	$C_2H_4Cl_2$ + iso-amylalcohol	various biol. mat.	S.P.	60
	3	as cetylpyridine-2				17
Pancuronium	1	rose-bengal	$CHCl_3$ + phenol (25%)	various biol. mat.	S.F.	52
	2	as choline-6				96
Piribenzyl®		as nicotine (Table 6)				10
Propantheline	1	as oxyphenonium-1				6
	2	tropaeoline oo	$CHCl_3$	urine	S.P.	78
	3	Na-9, 10-dimethoxy-anthracene-2-sulphonate	CH_2Cl_2	plasma	S.F.	108
	4	as cetylpyridine-2				17
Pyridostigmine	1	dipicrylamine, then I_2/I^-	CH_2Cl_2	plasma	S.P.	18
	2	bromothymol blue	CH_2Cl_2	water	S.F.	17
Secergan®	1	as desipramine-1 (Table 5)				33
	2	as amphetamine-3 (Table 4)				87
	3	as desipramine-5 (Table 5)				105
Suxamethonium		as choline-6				96
Thiamine		as amphetamine-3 (Table 4)				87
Thiazinamium	1	I^-	$C_2H_4Cl_2$	plasma, urine	G.L.C.	48
	2	bromophenol blue	monochlorobenzene	water	S.P.	20
d-Tubocurarine	1	as butylscopolamine				104
	2	as choline-6				96

1.6.5. Other drugs

Ion pair extraction is not only a very suitable method for separation and isolation of amines but also for *amino acids*. PERSSON (74) has described a column chromatographic method for the separation of some amino acids in water. Together with KARGER (76) the same author later described the application of high pressure liquid-liquid chromatography for separation of some amino acids (see also 1.4.2.).

For some *barbiturates* ion pair extraction has also been used successfully (12). Tetra-alkylammonium ions were used as counter ion and chloroform as organic phase. The spectrophotometric detection is not sensitive enough for the analysis of biological materials and far less sensitive than the gas chromatographic methods mentioned in the literature (*e.g.* BROCHMAN-HANSSEN and others, 14).

TABLE 8: *Ion pair extraction of various drugs*

drug		counter ion	extraction solvent or extraction method	medium	detection method	reference
A) *Amino acids*	1		column partition chromatography	water	S.P.	74
- dopa			Support: cellulose			
- methyldopa			Stat. phase: Cl^- or ClO_4^-			
- phenylalanine			Mob. phase: 1-pentanol + cyclohexane			
- tyrosine						
	2	as dopamine-2				76
B) *Barbiturates*		tetrapropyl- or tetrabutylammonium ion	$CHCl_3$	water	S.P.	12
- amobarbital						
- hexobarbital						
- phenobarbital						
C) *Penicillins*		tetrabutylammonium ion	$CHCl_3$	water	S.P.	68
a.o.:						
- benzylpenicillin						
- pheneticillin						
- phenoxymethyl-penicillin						
- propicillin						
D) *Sulphonamides*	1		column partition chromatography	water	S.P.	80
a.o.:			Support: diatomaceous earth			
- sulphadiazine			Stat. phase: tetrabutylammonium ion			
- sulphamerazine			Mob. phase: $CHCl_3$ or CH_2Cl_2			
- sulphamethazine	2		H.P.L.C. (partition chromatography)	water	S.P.	50
			Support: silica gel			
			Stat. phase: tetrabutylammonium ion			
			Mob. phase: butanol/hexane (25 : 75)			

Tetrabutylammonium ion can also be used as counter ion for the extraction of some *penicillins.*

Finally the comprehensive study by RADER (80) on the isolation of some *sulphonamides* deserves to be mentioned, in which again tetrabutylammonium was used as counter ion. The method is based on column partition chromatography. As mentioned earlier (1.4.2.) sulphonamides can also be separated by means of high pressure liquid-liquid chromatography in combination with ion pair partition (50).

In the last three methods the principles for the isolation of quaternary ammonium compounds have been inverted (see 1.6.4.). Now, large, polar molecules are extracted in the anion form with a large cation (tetrabutylammonium).

In Table 8 the methods mentioned in literature are summarized.

Conclusion

Ion pair extraction is a very useful method for the isolation and for the quantitative analysis of drugs in water, dosage forms or body fluids. The method is particularly suitable for drugs that are in the ionized form or can be easily converted to that state. Both batch extraction and liquid chromatographic procedures have been described. Especially gas chromatography and liquid chromatography offer many possibilities, because sophisticated apparatus can be used, which enables optimum adjustment of parameters and highly sensitive detection.

Much work has been done in the past concerning drugs, which contain an amine or an acid group. With the method of "extractive alkylation" it is possible to isolate other compounds, too.

References

1. APPLETON, H. D., B. N. LA DU, B. B. LEVY, J. M. STEELE and B. B. BRODIE (1953), *J. Biol. Chem. 205,* 803
2. AUERBACH, M. E. (1943), *Ind. Eng. Chem. Anal. Ed. 15,* 492
3. AUERBACH, M. E. (1944), *Ind. Eng. Chem. Anal. Ed. 16,* 739
4. BALLARD, C. W., J. ISAACS and P. G. W. SCOTT (1954), *J. Pharm. Pharmacol. 6,* 971
5. BERLIN, A., B. A. PERSSON and P. BELFRAGE (1975), *J. Pharm. Pharmacol. 25,* 466
6. BILES, J. A., F. M. PLAKOGIANNIS, B. J. WONG and P. M. BILES (1966), *J. Pharm. Sci. 55,* 909
7. BOON, P. F. G. and A. W. MACE (1969), *J. Chromatog. 41,* 105
8. BORG, K. O. (1969), *Acta Pharm. Suecica 6,* 425
9. BORG, K. O. (1970), *Acta Pharm. Suecica 7,* 673
10. BORG, K. O., H. HOLGERSSON and P. O. LAGERSTRÖM (1970), *J. Pharm. Pharmacol. 22,* 507

11. BORG, K. O. and A. MIKAELSSON (1970), *Acta Pharm. Suecica 7,* 673
12. BORG, K. O. and G. SCHILL (1968), *Acta Pharm. Suecica 5,* 323
13. BOURA, A. L. A. and A. MC. COUBREY (1962), *J. Pharm. Pharmacol. 14,* 647
14. BROCHMAN-HANSSEN, E. and T. OLAWUYI OKE (1969), *J. Pharm. Sci. 58,* 370
15. BRODIE, B. B. and S. UDENFRIEND (1945), *J. Biol. Chem. 158,* 705
16. BRODIE, B. B., S. UDENFRIEND and W. DILL (1947), *J. Biol. Chem.* 168, 335
17. CHATTEN, L. G. and K. O. OKAMURA (1973), *J. Pharm. Sci. 62,* 1328
18. COPER, H., G. DEYHLE and K. DROSS (1974), *Z. Klin. Chem. Klin. Biochem. 12,* 273
19. DESSOUKY, Y. M., B. A. MOUSA and H. M. NOUR EL-DIN (1974), *Pharmazie 9,* 577
20. DESSOUKY, Y. M., B. A. MOUSA and H. M. NOUR EL-DIN (1974), *Pharmazie 9,* 579
21. DIVATIA, G. J. and J. A. BILES (1961), *J. Pharm. Sci. 50,* 916
22. DOYLE, TH. D. and J. LEVINE (1967), *Anal. Chem. 39,* 1282
23. DOYLE, TH. D. and J. LEVINE (1968), *J. Assoc. Offic. Agr. Chemists 51,* 191
24. EHRSSON, H. (1971), *Acta Pharm. Suecica 8,* 113
25. EHRSSON, H. and A. TILLY (1973), *Anal. Letters 6,* 197
26. EKSBORG, S., P. O. LAGERSTRÖM, R. MODIN and G. SCHILL (1973), *J. Chromatog. 83,* 99
27. EKSBORG, S. and B. A. PERSSON (1971), *Acta Pharm. Suecica 8,* 205
28. EKSBORG, S. and B. A. PERSSON (1971), *Acta Pharm. Suecica 8,* 605
29. EKSBORG, S., B. A. PERSSON, J. VESSMAN and B. ENELL (1971), *J. Pharm. Sci. 60,* 475
30. EKSBORG, S. and G. SCHILL (1973), *Anal. Chem. 45,* 2092
31. ERVIK, M. and K. GUSTAVII (1974), *Anal. Chem. 46,* 39
32. GOTTLIEB, K. R. (1953), *Dansk Tidskr. Farm. 27,* 199
33. GRÖNINGSSON, K. (1970), *Acta Pharm. Suecica 7,* 635
34. GRÖNINGSSON, K., P. HARTVIG and L. MOLIN (1973), *Acta Pharm. Suecica 10,* 53
35. GUSTAVII, K. (1967), *Acta Pharm. Suecica 4,* 233
36. GUSTAVII, K. and G. SCHILL (1966), *Acta Pharm. Suecica 3,* 241
37. HADDOCK, L. A. and N. EVERS (1931), *Quart. J. Pharm. Pharmacol. 4,* 314
38. HIGUCHI, T. and J. I. BODIN (1961), In: *Pharmaceutical Analysis, p. 313,* Interscience, New York
39. HIGUCHI, T. and K. KATO (1966), *J. Pharm. Sci. 55,* 1080
40. HIGUCHI, T. and A. F. MICHAELIS (1968), *Anal. Chem. 40,* 1925
41. HIGUCHI, T., A. F. MICHAELIS, T. TAN and A. HURWITZ (1967), *Anal. Chem. 39,* 974
42. HODGMAN, C. D. (1973), *Handbook of Chemistry and Physics 54th Ed.,* The Chemical Rubber Co., Cleveland, Ohio
43. HUGOSSON, S., L. NYBERG and L. NILSSON (1972), *Acta Pharm. Suecica 9,* 249
44. IRWIN, G. M., H. B. KOSTENBAUDER, L. W. DITTERT, R. STAPLES, A. MISKER and J. V. SWINTOSKY (1969), *J. Pharm. Sci. 58,* 313
45. JANSSON, S. O., R. MODIN and G. SCHILL (1974), *Talanta 21,* 905
46. JENDEN, D. J., I. HANIN and S. J. LAMB (1968), *Anal. Chem. 40,* 125
47. JENNINGS, E. C. and H. MITCHNER (1967), *J. Pharm. Sci. 56,* 1590
48. JONKMAN, J. H. G. (1974), *Pharm. Weekblad 109,* 1095
49. JONKMAN, J. H. G., J. WIJSBEEK, S. HOLLENBEEK BROUWER-DE BOER, R. A. DE ZEEUW, L. E. VAN BORK and N. G. M. ORIE (1975), *J. Pharm. Pharmacol. 27,* 849
50. KARGER, B. L., S. C. SU, S. MARCHESE and B. A. PERSSON (1974), *J. Chromatogr. Sci. 12,* 678

51. KARLÉN, B., G. LUNDGREN, I. NORDGREN and B. HOLMSTEDT (1974), In: *Choline and Acetylcholine Handbook of Chemical Assay Methods* (I. Hanin, Ed.) 163–179, North-Holland Publishing Company, Amsterdam
52. KERSTEN, U. W., D. K. F. MEYER and S. AGOSTON (1973), *Clin. Chim. Acta 44*, 59
53. KRAUS, C. A. (1956), *J. Phys. Chem. 60*, 129
54. LAGERSTRÖM, P. O., K. O. BORG and D. WESTERLUND (1972), *Acta Pharm. Suecica 9*, 53
55. LEVINE, J. (1961), *J. Assoc. Offic. Agr. Chemists 44*, 285
56. LEVINE, J. (1962), *J. Assoc. Offic. Agr. Chemists 45*, 595
57. LEVINE, J. (1965), *J. Pharm. Sci. 54*, 485
58. LEVINE, J. and TH. D. DOYLE (1965), *J. Assoc. Offic. Agr. Chemists 48*, 608
59. LEVINE, J. and R. T. OTTES (1961), *J. Assoc. Offic. Agr. Chemists 44*, 291
60. LEVINE, R. and B. B. CLARK (1957), *J. Pharmacol. Exptl. Therap. 121*, 63
61. LINDÉN, E. and G. SCHILL (1967), *Acta Pharm. Suecica 4*, 327
62. MESSAGE OF THE LABORATORY OF THE DUTCH PHARMACISTS (1969), *Pharm. Weekblad 104*, 1247
63. MITCHNER, H. and E. C. JENNINGS (1967), *J. Pharm. Sci. 56*, 1595
64. MODIN, R. and S. BÄCK (1971), *Acta Pharm. Suecica 8*, 585
65. MODIN, R. and M. JOHANSSON (1971), *Acta Pharm. Suecica 8*, 561
66. MODIN, R. and G. SCHILL (1967), *Acta Pharm. Suecica 4*, 301
67. MODIN, R. and G. SCHILL (1970), *Acta Pharm. Suecica 7*, 585
68. MODIN, R. and M. SCHRÖDER-NIELSEN (1971), *Acta Pharm. Suecica 8*, 573
69. NYBERG, L. (1970), *J. Pharm. Pharmacol. 22*, 500
70. PERSSON, B. A. (1968), *Acta Pharm. Suecica 5*, 335
71. PERSSON, B. A. (1968), *Acta Pharm. Suecica 5*, 343
72. PERSSON, B. A. (1970), *Acta Pharm. Suecica 7*, 337
73. PERSSON, B. A. (1970), *Acta Pharm. Suecica 7*, 343
74. PERSSON, B. A. (1971), *Acta Pharm. Suecica 8*, 193
75. PERSSON, B. A. and S. EKSBORG (1970), *Acta Pharm. Suecica 7*, 353
76. PERSSON, B. A. and B. L. KARGER (1974), *J. Chromatog. Sci. 12*, 521
77. PERSSON, B. A. and G. SCHILL (1966), *Acta Pharm. Suecica 3*, 281
78. PFEFFER, M., J. M. SCHOR, S. BOLTON and R. JACOBSEN (1968), *J. Pharm. Sci. 57*, 1375
79. PFEFFER, M., J. M. SCHOR, N. GLUCK, M. S. SEMMEL and S. GRIBOF (1968), *J. Pharm. Sci. 57*, 36
80. RADER, B. R. (1973), *J. Pharm. Sci. 62*, 1148
81. REISS, R. (1956), *Arzneimittel-Forsch. 6*, 77
82. RINGBOM, A. (1963), In: *Complexation in Analytical Chemistry*, Wiley, (New York)
83. SANTORO, R. S. (1960), *J. Am. Pharm. Assoc. Sci. Ed. 49*, 666
84. SCHILL, G. (1959), *Anal. Chim. Acta 21*, 341
85. SCHILL, G. (1964), *Acta Pharm. Suecica 1*, 101
86. SCHILL, G. (1964), *Acta Pharm. Suecica 1*, 169
87. SCHILL, G. (1965), *Acta Pharm. Suecica 2*, 13
88. SCHILL, G. (1965), *Acta Pharm. Suecica 2*, 99
89. SCHILL, G. (1965), *Acta Pharm. Suecica 2*, 109
90. SCHILL, G. (1965), *Acta Pharm. Suecica 2*, 177

91. SCHILL, G. (1974), In: *Ion Exchange and Solvent Extraction*, Vol. 6, p. 1. J. A. Marinsky and Y. Markus Eds., Marcel Dekker, New York
92. SCHILL, G. and B. DANIELSSON (1959), *Anal. Chim. Acta 21*, 248
93. SCHILL, G. and M. MARSH (1963), *Svensk Farm. Tidskr. 67*, 385
94. SCHILL, G., R. MODIN and B. A. PERSSON (1965), *Acta Pharm. Suecica 2*, 119
95. SHAMSA, A. F. and R. H. MAGHSSOUDI (1976), *J. Pharm. Sci. 65*, 761
96. STEVENS, H. M. and A. C. MOFFAT (1974), *J. Forens. Sci. Soc. 14*, 141
97. SU, S. C., A. V. HARTKOPF and B. L. KARGER (1976), *J. Chromatog. 119*, 523
98. SYMPOSIUM ON ION PAIR PARTITION (Stockholm 24–26 October 1972), *Acta Pharm. Suecica 9*, 609
99. THOMIS, G. N. and A. Z. KOTIONIS (1956), *Anal. Chim. Acta 14*, 11
100. TILLY, A. (1973), *Acta Pharm. Suecica 10*, 111
101. TSUBOUCHI, M. (1971), *J. Pharm. Sci. 60*, 943
102. VESSMAN, J. and S. STRÖMBERG (1969), *Acta Pharm. Suecica 6*, 505
103. VESSMAN, J., S. STRÖMBERG and G. RIETZ (1970), *Acta Pharm. Suecica 7*, 363
104. VIDIC, H. J., H. DROSS and H. KEWITZ (1972), *Z. Klin. Chem. Klin. Biochem. 10*, 156
105. WAHLUND, K. G. and K. GRÖNINGSSON (1970), *Acta Pharm. Suecica 7*, 615
106. WESTERLUND, D. and K. O. BORG (1970), *Acta Pharm. Suecica 7*, 267
107. WESTERLUND, D., K. O. BORG and P. O. LAGERSTRÖM (1972), *Acta Pharm. Suecica 9*, 47
108. WESTERLUND, D. and K. H. KARSET (1973), *Anal. Chim. Acta 67*, 99
109. WOODS, L. A., J. COCHIN, E. J. FORNEFELD, F. G. MCMAHON and M. H. SEEVERS (1951), *J. Pharmacol. Exptl. Therap. 101*, 188

2

Determination of thiazinamium cations in body fluids

Contents

Summary

A sensitive and selective method for quantitative determination of thiazinamium methylsulphate in plasma, urine, bile and saliva is described. The procedure is based on ion pair extraction of the quaternary ammonium compound with iodide as counter ion. Optimum extraction conditions have been established on the basis of the extraction constant and the constants for side reaction. The ion pair extraction is followed by gas chromatography with alkali flame ionization detection. Thiazinamium sulphoxide, which was found to be the only metabolite of thiazinamium in man, does not interfere in the present procedure.

2.1. Introduction

In Part I, Chapter 1.1. it was mentioned that thiazinamium methylsulphate belongs to the group of phenothiazine derivatives. It takes up a special position in this series because of the quaternary ammonium group present in the side chain of the molecule. This causes an alteration of the physical, chemical, physico-chemical as well as the pharmacological properties as compared to its tertiary analogue promethazine (mostly used as the hydrochloride, which is also known under the trade name Phenergan®).
Most phenothiazine derivatives are tertiary, some secondary amines; they have weakly basic properties.
For isolating phenothiazine drugs from body fluids and their subsequent quantitative determination several methods are available in the literature, *e.g.* DRISCOLL and others, 1964; GUDZINOWICZ and others, 1965; JOHNSON and others, 1965; KOFOED and others, 1966; CURRY and BRODIE, 1967; JAIN and KIRK, 1967; CURRY, 1968; HAMMAR and HELMSTEDT, 1968; HAMMAR and others, 1968; CURRY and MOULD, 1969; FLINT and others, 1971; JACKSON, 1971; KELSEY and MOSCATELLI, 1973; RIVERA-CALIMLIM and others, 1973; CURRY, 1974; DE LEENHEER, 1974; EASSIEN and others, 1975; LARSEN and NAESTOFT, 1975; VANDERHEEREN and THEUNIS, 1976. Most of these are based on isolation of the drugs from the biological medium in their unionized form. This can be performed by increasing the pH of the aqueous medium to 9–10. The unionized amines (free bases) are then extracted from this milieu by shaking with a lipophilic organic solvent such as hexane, heptane or toluene to which a few percent iso-amylalcohol has been added in order to avoid adsorption to the glass wall of the extraction tubes. In general one or more re-extractions are applied to clean up the extract. Gas chromatography is the most commonly used method for further separation and for quantitation. Often electron capture detection or detection by means of mass fragmentometry is used. (*N.B.* Studies on the gas chromatographic behaviour of phenothiazine drugs have been published by *e.g.* MARTIN and others, 1963 and DE LEENHEER, 1973).
A review of several methods of analysis for phenothiazine drugs in biological materials has been given by CIMBURA (1972), which includes spectrophotometry, spectrofluorimetry, thin-layer and gas chromatography.
There are hardly any methods to be found in literature for the determination of thiazinamium methylsulphate. Although a few methods have been given for determinating thiazinamium in bulk substance, dosage forms or aqueous solutions (see Part I, Chapter 4) these methods have two drawbacks.
On one hand the procedures do not include an isolation step, which is required with biological fluids and on the other hand the sensitivity is rather

low (milligram or microgram range).
One of the differences between thiazinamium methylsulphate and the non-quaternary phenothiazine derivatives is its large water solubility (about 150 mg/ml) at all pH values, due to the fact that it is completely ionized under all pH conditions. So, the general methods for the isolation of phenothiazine derivatives described earlier, are inadequate in the case of thiazinamium methylsulphate. However, several research workers did not realize this and described methods for the isolation of thiazinamium methylsulphate from body fluids which are based on extraction of the unionized form that they believe would be formed at alkaline pH values (BERTI and CIMA, 1954; KLEINSORGE, THALMAN & others, 1959; KERCKHOFFS and HUIZINGA, 1967). As this is not the case, their procedures must be considered with doubt.
Because of the dominant influence of the quaternary ammonium group on the physico-chemical properties of the thiazinamium molecule as a whole, applicability was considered of methods occurring in literature for isolation of other quaternary ammonium compounds from body fluids (or in general from aqueous solutions).
As has been described extensively in Part II, Chapter 1, more recent studies have shown that two possibilities seem to exist for the isolation of quaternary ammonium compounds from aqueous medium, namely dealkylation (and subsequent extraction of the unionized tertiary amine) and ion pair extraction.
Dealkylation, though very useful as such, has several practical drawbacks when working with biological materials such as time consuming methods, high temperatures, *etc.* (JENNINGS and MITCHNER, 1967; MITCHNER and JENNINGS, 1967; JENDEN and others, 1968).
The alternative method of ion pair extraction is however very useful for the isolation of quaternary ammonium compounds from biological material, its main advantage being the great universality in addition to a wide variety of possibilities for selection of optimum conditions. In fact, the latter seems to be the preferable method for such compounds. In general (see Part II, Chapter 1, Table 7) spectrophotometry or spectrofluorimetry is used for quantitative determination of the ion pair after the extraction has been completed. These methods are useful for plasma concentrations in the microgram range, but they are usually not sensitive enough for quantitative determination of therapeutic plasma concentrations of *e.g.* parasympatholytic drugs (low nanogram range). Thus, for example the method of determinating thiazinamium methylsulphate in plasma, which is described in the leaflet MULTERGAN®, *Note technique* (1961) is insufficiently sensitive. This method is based on ion pair extraction with methyl orange as counter ion followed by spectrophotometric measurement. The detection limit is 3.3 μg/ml plasma. Other disadvantages of spectrometric analyses are the lack of specificity and the susceptibility to interference from other

substances. This necessitates extensive clean up procedures in order to avoid high blank values. ALLGÉN, EKMAN and others (1960) reported that they did not succeed in determining the quaternary phenothiazine compound Secergan® in plasma when using a combination of ion pair extraction and spectrophotometry. The use of a gas chromatographic procedure would yield a higher selectivity and sensitivity, especially when using element specific detectors.
Although quaternary ammonium compounds are difficult to handle in gas chromatography it was felt that in the case of thiazinamium these problems could be overcome and we decided to try to develop a method for the determination of thiazinamium methylsulphate in body fluids, which is based on ion pair extraction followed by gas chromatography.

2.1.1. Ion pair extraction of thiazinamium cations from water

When using an ion pair extraction method for the isolation of thiazinamium cations from body fluids, various conditions have to be met. Because we had selected a gas chromatographic method for further separation and detection this meant the addition of an important restriction. In summary the following factors should be taken into account:
a. the ion pair extraction has to be quantitative
b. the ion pair extraction has to be selective
c. the ion pair must be stable
d. the ion pair has to be detectable by means of gas chromatography

ad a. The ion pair extraction has to be quantitative
The extraction constant for the ion pair extraction should be high enough to ensure a quantitative yield. This means that side reactions causing loss of thiazinamium (*e.g.* association in the aqueous phase) should be absent.

ad b. The ion pair extraction has to be selective
The extraction method should have adequate selectivity so that no extensive clean up procedures are required which in general are time consuming and may give rise to low recoveries. Optimum extraction conditions can be obtained by the correct choice of counter ion, extraction solvent and pH. (*N.B.* pH does not influence the extraction of thiazinamium cation, but of course it may affect the extraction so that it is accompanied by some co-extraction of interfering substances).

ad c. The ion pair must be stable
No degradation or oxidation should occur during the procedure. Because phenothiazines (including thiazinamium) are liable to oxidation (light, oxygen) precautions should be taken to avoid this.

ad d. The ion pair has to be detectable by means of gas chromatography
Application of gas chromatography is only possible if the sample can easily be transferred into the gaseous phase and if no on-column decomposition or irreversible column adsorption occurs. Because quaternary ammonium ions are not volatile as such, this requires counter ions that allow application of gas chromatography.

Several anions can be considered for ion pair formation with quaternary ammonium compounds (see also Part II, Chapter 1, Table 1). We tested a great variety of counter ions, using extraction solvents from the following series: carbon tetrachloride, chloroform, dichloromethane, 1,2-dichloroethane, benzene, toluene, cyclohexane, n-hexane, iso-amylalcohol, diethyl ether, ethyl acetate, methyl-iso-butylketone. Moreover mixtures of n-hexane (and toluene) with 1-propanol and iso-amylalcohol in various ratios were used. The counter ions were both inorganic and organic in kind. Of the inorganic ions we tested: Cl^-, Br^-, I^-, CN^-, CNS^- and ClO_4^-. Of the organic ones we tested some phenols (phenol, picric acid, 2,4-dinitro-1-naphtol), some carboxylic acids (acetic acid, phenylacetic acid, benzoic acid, salicylic acid), some sulphonic acids (benzene-sulphonic acid, toluene-4-sulphonic acid, naphtalene-2-sulphonic acid, anthracene-4-sulphonic acid), some acid dyes (methyl orange, bromothymol blue, hydroxy-naphtol blue) and finally dipicrylamine.
For all counter ions the ion pair formation with thiazinamium and subsequent extraction with the solvents mentioned above was studied. In addition, the gas chromatographic behaviour of the various ion pairs was tested (see below).
The acids were used as sodium salts at pH = 6.5 to give sufficient amounts of ions and to avoid oxidation of thiazinamium in acid milieu. Of the organic anions especially benzene-sulphonic acid and toluene-4-sulphonic acid proved to be useful counter ions because they gave well extractable ion pairs (particularly with chloroform). These ion pairs also were gas chromatographically detectable.
Of the inorganic anions especially iodide and perchlorate met with both requirements.
Of these four counter ions (benzene-sulphonic acid, toluene-4-sulphonic acid, iodide and perchlorate) the inorganic ones were more suitable than the organic ones, because the latter showed a tendency to cause oxidation during the extraction procedure (solution turned slightly red). Therefore our tests were continued with iodide and perchlorate. The chlorinated hydrocarbons chloroform, dichloromethane and 1,2-dichloroethane, like for ion pairs in general (Part II, Chapter 1), showed to be the best extraction solvents of the series mentioned above.

Determination of extraction constants of thiazinamium

For these solvents we determined the extraction constants and the constants for side reactions for ion pair extraction of thiazinamium cations from aqueous solutions. To this end we used the method described by MODIN and SCHILL (1970). Thiazinamium methylsulphate was dissolved in a buffer solution. In most cases a sodium phosphate buffer of pH = 6.5, ionic strength 0.1 was used.

In one case (see Table 1) extractions were also carried out at pH = 10.0. Always the concentration of thiazinamium methylsulphate in the aqueous solution was appr. 1×10^{-4} mol/l and the extractions were done with six different solutions of the counter ion with concentrations which vary from appr. 1×10^{-3} to appr. 1×10^{-2} mol/l (The exact concentrations are included in Tables 1 and 2).

Procedure: To 10.0 ml of the aqueous buffered solution of thiazinamium methylsulphate 10.0 ml of the aqueous solution of the counter ion was added in a centrifugation tube (glass with Quickfit® stopper). This aqueous phase was shaken with 20.0 ml of the organic solvent for 30 min. at 25° C in a thermostated water bath (n = 100 times per min.). The organic phase had been equilibrated with buffer solution before use. After centrifugation (10 min. at g = 2000) the phases were separated with a capillary siphon. The concentration of the ion pair in the organic phase and of the cation in the aqueous phase were determined from the absorbances at 254 nm in the case of perchlorate. Using iodide, absorbance was measured at the second maximum at 303 nm, because of interference of iodide ion at a lower wavelength. All reagents were of Pro Analysi® grade. Iodide was used in the form of potassium iodide and perchlorate as the sodium salt. The extraction solvents were of Uvasol® (spectrophotometric grade (E. Merck, Darmstadt, G. F. R.)). Spectrophotometric measurements were carried out with a Zeiss Spectrophotometer P.M.Q.II.

Extraction constants were calculated from equation III (see Part II, Chapter 2), which can be modified for thiazinamium as follows:

$$E^*_{ThX} = \frac{c'_{ThX_{org}}}{c'_{Th^+_{aq}} \times c'_{X^-_{aq}}} \qquad \text{(IX)}$$

In which:

E^*_{ThX} = the conditional extraction constant for thiazinamium.

$c'_{Th^+_{aq}}$ = the concentration of thiazinamium cations in the aqueous phase after equilibrium has been obtained.

$c'_{X^-_{aq}}$ = the concentration of anions (X is I^- or ClO_4^-) in the aqueous phase, after equilibrium has been obtained.

$c'_{ThX_{org}}$ = the concentration of thiazinamium present in the organic phase as ion pairs with X (X is I^- or ClO_4^-).

In the course of these activities it was found that the conditional extraction constant varies with the concentration of the counter ion added. In fact it was found that E^*_{ThX} increased with decreasing values of $([Th^+_{aq}] \times c'_{X^-_{aq}})$. BORG (1969) showed that this is an indication of a side reaction occurring, namely dissociation of the ion pair in the organic phase. In such cases the extraction constants and the dissociation constant can be calculated from the following equation (see also equation VIII in Part II, Chapter 1):

$$E^*_{ThX} = E_{ThX} + (E_{ThX} \times K_{diss})^{\frac{1}{2}} \times ([Th^+_{aq}] \times c'_{X^-_{aq}})^{-\frac{1}{2}} \qquad (X)$$

In Table 1 the extraction and dissociation constants of thiazinamium iodide are given for various solvents. Dissociation of the ion pair in the organic phase results in a better efficacy of the extraction, especially with low initial concentrations of thiazinamium cations. The table shows that in the case of 1,2-dichloroethane substantial dissociation occurs. Although the highest extraction constant E_{ThI} is obtained with dichloromethane, the value of the

TABLE 1: *Extraction and dissociation constants of thiazinamium iodide in different solvents; pH of the aqueous phase is given.*

	chloroform	dichloromethane	1,2-dichloroethane	
	pH = 6.5	pH = 6.5	pH = 6.5	pH = 10.0
$[Th^+_{aq}]$	9.9×10^{-5}	9.9×10^{-5}	9.9×10^{-5}	9.9×10^{-5}
$c'_{I^-_{aq}}$	9.5×10^{-4} to 9.5×10^{-3}	9.5×10^{-4} to 9.5×10^{-3}	9.5×10^{-4} to 9.5×10^{-3}	9.5×10^{-4} to 9.5×10^{-3}
$c'_{ThI_{org}}$	9.3×10^{-6} to 4.7×10^{-5}	2.8×10^{-5} to 7.4×10^{-5}	1.3×10^{-5} to 6.8×10^{-5}	2.5×10^{-5} to 6.6×10^{-5}
E	0.99×10^2	2.88×10^2	1.22×10^2	1.00×10^2
log E	1.99	2.46	2.09	2.00
K_{diss}	*	2.4×10^{-6}	7.3×10^{-5}	8.1×10^{-5}
log K_{diss}	*	−5.6	−4.1	−4.1

* Value for K_{diss} in chloroform is negligible

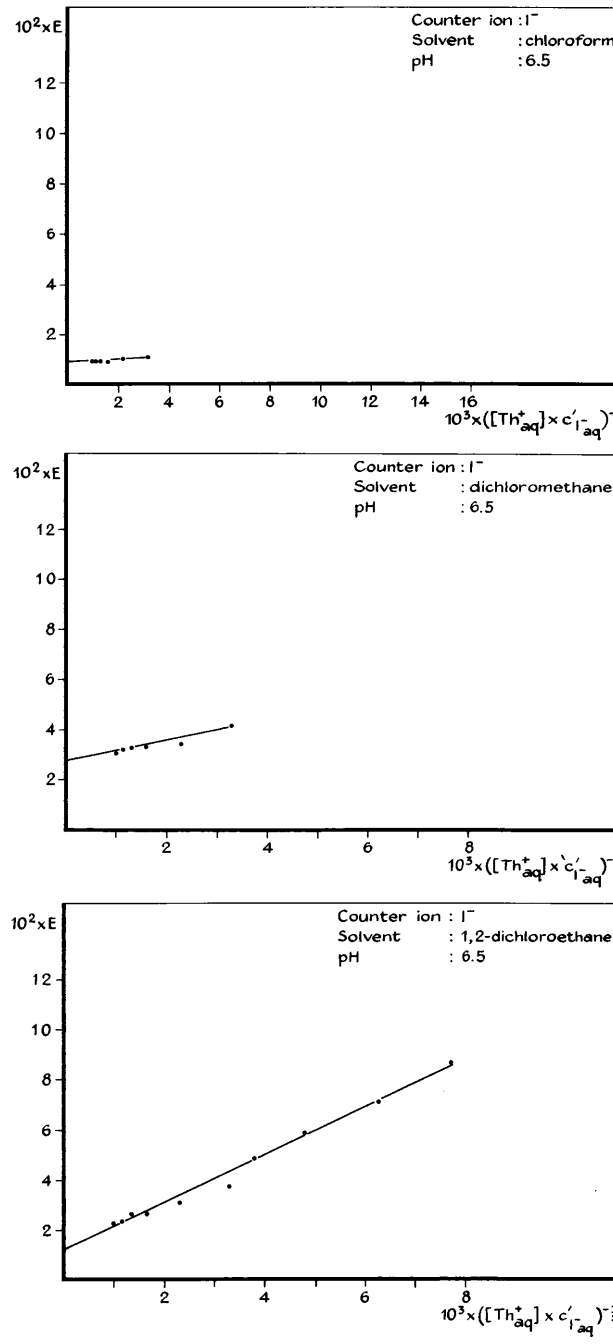


FIG. 1: *Graphical representation of extraction and dissociation constants of thiazinamium iodide. From this plot the extraction constant E is given by the intercept of the y-axis (Note, that the scale of the y-axis is different of that of Fig. 2.). The slope of the curve is an indication of the degree of dissociation. The organic phase is chloroform, dichloromethane and 1,2-dichloroethane respectively. The pH of the aqueous solution is 6.5.*

conditional extraction constant E^*_{ThI} in the range of 50–500 ng/ml is considerably higher when 1,2-dichloroethane is used. Thus for a concentration of thiazinamium of approximately 5×10^{-7} mol/l (which equals a therapeutic plasma level) and $c'_{I^-_{aq}} = 1.8 \times 10^{-2}$, $\log E^* = 2.5$ in dichloromethane but 3.1 in 1,2-dichloroethane. For the third organic phase, chloroform, it was found that the conditional extraction constant was independent of the concentration of the ions. In other words $E^*_{ThI} = E_{ThI}$ which means that no side reactions occur. These phenomena are graphically represented in Fig. 1 by plotting E^*_{ThI} (y-axis) versus $([Th^+_{aq}] \times c'_{I^-_{aq}})^{-\frac{1}{2}}$ on the x-axis. The intercept of the y-axis will give the value of E_{ThI} (see the above equation). As regards the case of chloroform, the curve is parallel to the x-axis, which means that in chloroform no dissociation of the ion pair thiazinamium iodide occurs. With dichloromethane E^*_{ThI} increases as the value of $([Th^+_{aq}] \times c'_{I^-_{aq}})^{-\frac{1}{2}}$ increases, which means that there is some, but little, dissociation of the ion pair thiazinamium iodide in this liquid. With 1,2-dichloroethane, however, E^*_{ThI} increases strongly with increasing value of $(Th^+_{aq} \times c'_{I^-_{aq}})^{-\frac{1}{2}}$), which points to considerable dissociation.
In Table 2 the extraction and dissociation constants for the thiazinamium perchlorate ion pair in various extraction solvents are given. It will be noticed that in this concentration range the extraction constants for

TABLE 2: *Extraction and dissociation constants of thiazinamium perchlorate in different solvents; pH of aqueous phase is 6.5.*

	chloroform	dichloromethane	1,2-dichloroethane
$[Th^+_{aq}]$	9.9×10^{-5}	9.9×10^{-5}	9.9×10^{-5}
$c'_{ClO_{4_{aq}}^-}$	4.7×10^{-4} to 9.5×10^{-5}	9.5×10^{-5} to 9.5×10^{-4}	4.8×10^{-5} to 4.7×10^{-4}
$c'_{ThClO_{4_{org}}}$	6.5×10^{-5} to 9.0×10^{-6}	2.6×10^{-5} to 7.6×10^{-5}	2.3×10^{-5} to 7.3×10^{-5}
E	2.05×10^2	3.27×10^3	5.59×10^3
log E	2.31	3.51	3.75
K_{diss}	-	7.8×10^{-6}	1.5×10^{-5}
log K_{diss}	-	−5.1	−4.8

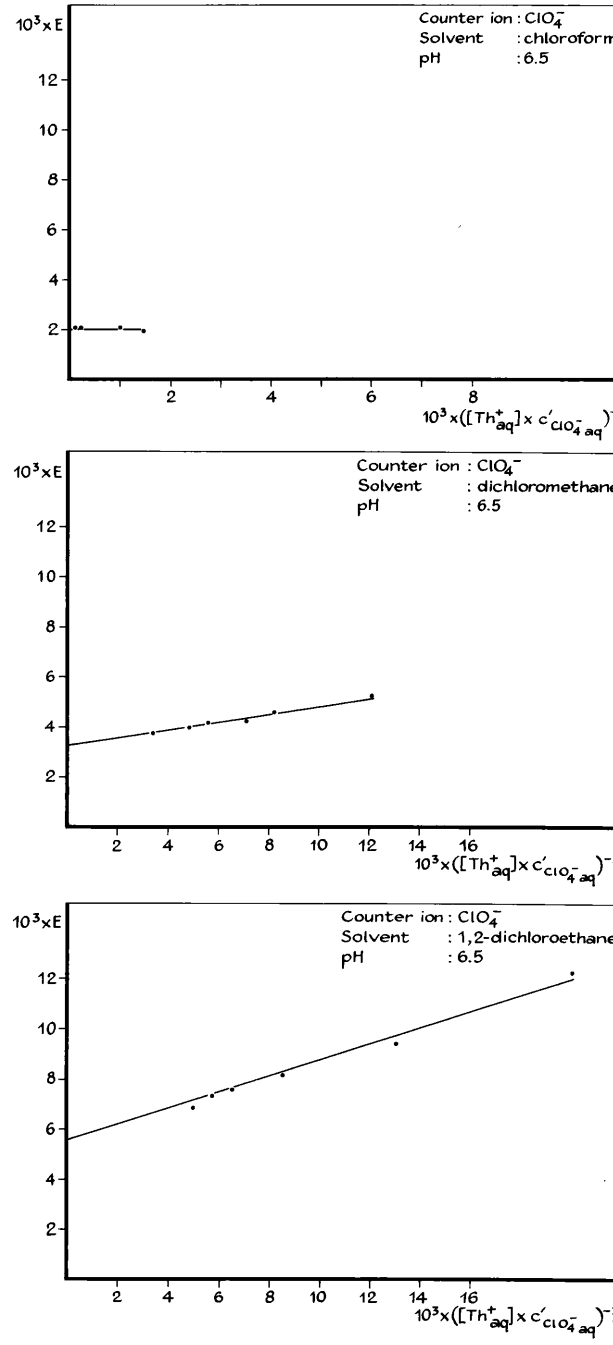


FIG. 2: *Graphical representation of extraction and dissociation constants of thiazinamium perchlorate. From this plot, the extraction constant E is given by the intercept of the y-axis (Note, that the scale of the y-axis is different from that in Fig. 1.). The slope of the curve is an indication of the degree of dissociation. The organic phase is chloroform, dichloromethane and 1,2-dichloroethane respectively. The pH of the aqueous solution is 6.5.*

perchlorate are higher than for iodide. On the other hand the constants for dissociation of the ion pair in the organic phases are lower than if iodide is used: again there is no dissociation in chloroform, very slight dissociation in dichloromethane and some dissociation in 1,2-dichloroethane (see Fig. 2). With the same concentrations as given in the example for iodide, the log-value for the calculated conditional extraction constant for extraction with perchlorate as a counter ion is 3.7 for dichloromethane and 3.9 for 1,2-dichloroethane. So, it can be concluded, that extraction of thiazinamium cations from water using perchlorate as a counter ion is more effective than iodide, particularly when dichloromethane and 1,2-dichloroethane are employed as organic phase. However, with iodide as a counter ion extraction constants are still acceptable for quantitative work.

2.1.2. Gas chromatography of thiazinamium ion pairs

Specific difficulties with the gas chromatographic determination of thiazinamium can be summarized as follows:

a. thiazinamium is a quaternary ammonium ion, and is not volatile as such;
b. thiazinamium is a phenothiazine derivative, so irreversible column adsorption may occur;
c. thiazinamium is given in low doses (*e.g.* intramuscular administration) to patients, so low plasma levels may be expected.

ad a. Thiazinamium is a quaternary ammonium ion and is not volatile as such

Since only volatile components can be analyzed by means of gas chromatographic methods, thiazinamium cation must be converted into a volatile derivative.

Most methods of derivatization require a reactive group in the molecule of the sample, as for example has been mentioned in the article reviewing derivatization in gas chromatography by AHUJA (1976). Unfortunately, thiazinamium does not contain such reactive groups. The only derivatization reaction available is demethylation to a tertiary analogue. Methods for demethylation of quaternary ammonium compounds have been discussed in Part II, Chapter 1, Section 1.6.4. As stated these methods have several drawbacks, when biological materials and/or chemically unstable samples are being employed. For that reason this method had better be discarded when thiazinamium methylsulphate is to be determined in body fluids. As ion pair extraction had been selected for isolation of quaternary ammonium compounds, we searched for an ion pair which could also be subjected to gas chromatographic analysis.

In literature only a few examples of direct gas chromatographic analysis of

ion pairs can be found (see also Part II, Chapter 1.5). BOON and MACE (1969) extracted some tertiary amines (among them chlorpromazine) with bromothymol blue as counter ion and chloroform as extraction solvent. The ion pairs were split into the amines in the gas chromatograph. Being volatile, the tertiary amines could be analyzed by gas chromatography. Our attempts to isolate thiazinamium in the same way with bromothymol blue were successful, but the ion pair could not be determined by means of gas chromatography. Most of the ion pairs of thiazinamium with large organic counter ions proved to be not suitable to gas chromatographic analysis either (except benzene-sulphonic acid and toluene-4-sulphonic acid). VIDIC and others (1972) described isolation of some quaternary ammonium compounds as "Jod-Komplex" (formed after addition of a mixture of I_2 and KI) with chloroform as extraction solvent. The ion pairs (of *e.g.* d-tubocurarine, methylcurarine, neostigmine and butylscopolamine) were split in a special pyrolysis oven (800° C) and the alkyliodide which was formed was detected by means of a flame ionization detector (detection limit approximately 1 µg/ml).

At the time of publication of VIDIC's paper, we had already found, that addition of potassium iodide alone was sufficient for ion pair formation with thiazinamium cation to take place, and that isolation could be performed by extraction with aliphatic halogenated hydrocarbons.

When we subjected the ion pair thiazinamium iodide and also thiazinamium perchlorate to a gas chromatographic analysis, it proved that both could be successfully determined in this way. For practical reasons (less co-extraction of disturbing compounds from plasma, greater reproducibility in the gas chromatographic analysis) we decided to focus our attention on the iodide ion pair. When an extract containing thiazinamium iodide was injected into our gas chromatographic system (see 2.2) beside the solvent peak one sharp peak appeared (Fig. 3). Gas chromatography-mass spectrometry showed this peak to be the tertiary amine promethazine, which suggests that thiazinamium iodide is demethylated in the injection port of the gas chromatograph (300° C). Investigations with mass spectrometry proved, that already at temperatures of about 250° C instantaneous and complete demethylation into promethazine and methyliodide occurs. As methyliodide is very volatile (boiling point 42.5° C) it appears in the solvent peak when analyzing according to our system (oven temperature 240° C). Then a temperature of the injection port of 300° C was found to be sufficiently high to complete the process of demethylation and no special pyrolysis oven with its very high temperature (800° C) was necessary. VIDIC and associates (*l.c.*) detected the alkyliodide that is split off, after a chromatographic process at low oven temperatures (between 50° and 90° C). On the contrary we prefer to determine the tertiary amine that is formed, because detection of an alkyliodide is not very specific when also other quaternary ammonium compounds occur in the medium. Further-

more, formation of promethazine enables us to use a detector that is selective for nitrogen (see below), which results in a much higher sensitivity of appr. 2 ng/ml for our method against appr. 1 μg/ml for the method described by VIDIC. As an additional advantage of detecting the amine instead of the alkyliodide, application of relatively high oven temperatures does not cause column contamination.

ad b. Thiazinamium is a phenothiazine derivative, so column adsorption may occur

It is known from literature (AHUJA, 1976) that irreversible column adsorption may occur with phenothiazine derivatives (as with tertiary amines in general).

We indeed observed that promethazine, especially at low concentrations (nanogram range) became partly adsorbed to the column. Therefore several precautions had to be taken to reduce loss of sample to a minimum. As is usual in bioanalysis of chemically unstable drug molecules we used glass columns. Also glass-lined injection ports and glass-lined detector entrances were used. To avoid adsorption to the glass wall new empty columns were treated with H.M.D.S. (= hexamethyldisilazane, Pierce Chemical Company) during 48 hours at room temperature.

The choice of support and stationary phase is important too. Chromosorb G- (A.W.-D.M.C.S.)-High Performance 80–100 mesh (Johns-Manville) proved to be very useful as support. As it has been treated with D.M.C.S. (= dimethylchlorosilane) this material is very inert. In practice, we found that after using the column for a prolonged period it was necessary to perform a resilylation. Obviously H.M.D.S. and D.M.C.S. were washed off for a great deal. We found Silyl-8® G.L.C. column conditioner (Pierce Chemical Company) to be a suitable resilylation agent, especially when injected into the gas chromatograph frequently (1–2 times a day) and in small portions.

For this type of compounds weakly polar silicone polymers are advisable as stationary phase. We tested columns containing Chromosorb-G.H.P. coated with the following stationary phases: OV-1 (3%); OV-7 (1%); OV-17 (3%); SP-2250 (1, 2 and 3%) and SP-2100 (3%). When using stationary phases of the OV-series some loss due to irreversible column adsorption was found. SP-2250 and SP-2100 were better, especially SP-2100, which by consequence was selected for all further experiments (3% coating).

ad c. Thiazinamium is given in low doses (e.g. intramuscular administration) to patients, so low plasma levels may be expected

Although no data from literature were available about plasma concentrations of thiazinamium methylsulphate, the low parenteral doses (12.5–25.0 mg) are indicative of plasma concentrations in the sub-microgram range. Detection of such low amounts of a drug requires highly sensitive detectors.

In general flame ionization detectors do not have sufficient sensitivity to detect such low concentrations. Element specific detectors like electron capture, flame photometer and alkali flame ionization detectors can provide for a higher selectivity and sensitivity (see the review by NATUSH and THORPE, 1973). Electron capture detectors give high responses to molecules that contain a high percentage of halogen atoms. Often samples are converted to fluor containing derivatives, but this conversion requires reactive groups in the molecule ($-OH$; $-NH_2$; $-COOH$ or $-C=$). However, the thiazinamium molecule does not contain such reactive groups. Flame photometer detectors are particularly suitable to the detection of compounds containing phosphorus or sulphur (SUGIYAME and others, 1973 a, b, c).

Although suitable for thiazinamium (containing a sulphur atom), employment of the flame photometer detector has a few practical disadvantages (restricted sensitivity, quickly contaminated, rather difficult to handle). On the other hand, several papers in the literature report about successful application of an alkali flame ionization detector (A.F.I.D.) in the analysis of nitrogen containing compounds in biological fluids (DONIKE and others, 1970; JAMES and WARING, 1971; RIEDMANN, 1972a; BILZER and GUNDERTREMY, 1973; MEFFIN and others, 1973; RIEDMANN, 1973; BREIMER, 1974; BREIMER and VAN ROSSUM, 1974; RIEDMANN, 1974b; HUCKER and STAUFFER, 1976).

This type of detector is also called nitrogen flame ionization detector (N-F.I.D.) or "nitrogen detector". Apart from the high sensitivity for nitrogen atoms this detector is also sensitive for phosphorus and, at a lower degree, for sulphur atoms.

Since KARMEN (1964) and KARMEN and GIUFFRIDA (1964) described a modified flame ionization detector selective for phosphorus, which was based on the thermionic principle, a wide variety of detector designs has been evaluated (SWAN, 1972; HARTIGAN and others, 1974; KOLB and BISCHOFF, 1974; MAIER-BODE and RIEDMANN, 1975). AUE and others (1967) were the first to report about the sensitivity of thermionic detectors to nitrogen in organic molecules. Most alkali flame ionization detectors consist of a crystal of an alkali salt (particularly caesium and rubidium are used as bromide or sulphate). The design selected for introducing the alkali vapour into the flame (see below) and the type of alkali salt used influences the detector sensitivity and selectivity by several orders of magnitude. In our case (a Hewlett Packard High Sensitivity Nitrogen Detector model 15161-B) a crystal of rubidium bromide was used. A rubidium bromide crystal is grown from the molten salt and machined to form a cylinder with a central bore in its longitudinal axis. The crystal fits into the cylindrical collector electrode. A mechanical means is provided for easy adjustment of the height of the crystal above the flame jet in order to select the desired position of high sensitivity and high selectivity operation. A platinum loop

gate electrode is mounted concentrically with the jet top, enhancing the signal/noise ratio by up to a factor of ten when a corresponding negative potential is applied.

MAIER-BODE and RIEDMANN (1975), who wrote an excellent review about alkali flame ionization detectors in general and in particular about the Hewlett Packard model 15161-B, proposed the following reaction mechanism. The rubidium bromide evaporates and rubidium atoms are formed by the presence of active hydrogen at a flame temperature of about 600° C. When nitrogen (or phosphorus, sulphur) is introduced in the flame, it will react with the alkali metal atoms to form alkali ions which cause the detector signal. However, other mechanisms were proposed by other scientists (KARMEN, 1964; CREMER, 1967; JANAK and others, 1968).

The alkali flame ionization detector responds proportionally and selectively to the percentage of nitrogen which the drug contains; this response is independent of the chemical structure (RIEDMANN, 1974b). This author demonstrated that for several drugs, among them various phenothiazine derivatives, an A.F.I.D. is approximately 100 times more sensitive than a normal F.I.D.

For thiazinamium (which arrives in the detector as promethazine) containing two nitrogen atoms a good sensitivity could be expected. We actually found that the A.F.I.D. is up to 50 times more sensitive than a normal F.I.D. Apart from this higher sensitivity the selectivity of the detector is of great value in bioanalysis. Firstly, it lowers the detection limits because the detector is rather insensitive to bleeding of the liquid phase, even at elevated column temperatures (in our case 240° C). Bleeding is often the limiting factor in determining the detection limit with an F.I.D. Secondly, the solvent appears as a sharp peak with little or no tailing. Thirdly, the detector is insensitive for co-extracted interferences such as lipids so that in many cases no difficult, time-consuming sample clean-up is necessary and a single-step extraction can be used (RIEDMANN, 1972a, 1974b; BREIMER, 1974). A disadvantage of an A.F.I.D. containing a rubidium bromide crystal is that its sensitivity decreases when large quantities of halides are present in the sample (*e.g.* chlorine-containing solvents). Chloride ions formed by flame ionization give a rubidium chloride film on the crystal surface, which reduces the amount of rubidium bromide that can evaporate. Then, after elution of a halogenated solvent the baseline slowly recovers to its original value. In our case this means that the solvents which gave the highest extraction constants for the ion pair thiazinamium iodide (chloroform, dichloromethane, 1,2-dichloroethane) cannot be injected into the gas chromatograph directly. So, an additional step to remove the halogenated hydrocarbon is required (see also below, under plasma extractions). The presence of iodine in the molecule, results in introduction of methyliodide into the detector may be an explanation for the fact that in our procedure the A.F.I.D. is only some 50 times more sensitive than the F.I.D. RIEDMANN

(1974b) also described a 50 times greater sensitivity when analyzing phenothiazines containing a chlorine atom (*e.g.* chlorpromazine). For phenothiazines without chlorine atom the sensitivity was said to increase at least a 100 times.

Optimal use of an A.F.I.D. requires exact adjustment of several parameters. All parameters inducing a rise in temperature of the crystal surface and causing rubidium bromide to evaporate into the detector flame reaction zone influence the output signal and the selectivity; they include hydrogen flow, air flow and carrier gas flow rates, detector base temperature, crystal position and jet internal diameter. Optimum hydrogen flow rates should be selected between 28 and 32 ml/min. Stability of the flow rate of hydrogen gas is very important. In order to keep the nitrogen output signal constant within ± 1%, the hydrogen flow rate has to be held stable within ± 0.2%. It is recommended to set the air flow rate to the lowest value that will keep the flame burning (180–220 ml/min.). By reducing the air flow the detector selectivity is increased by suppressing the hydrocarbon signal. In order to keep the nitrogen signal constant within ± 1% the air flow rate has to be kept constant within 1.5%. Helium is recommended as carrier gas. In order to obtain adequate separation of the sample one should select a carrier gas flow rate which ensures a sufficient number of theoretical plates. Usually, this is a carrier gas flow rate of between 30 and 50 ml/min. However, the alkali flame ionization detector gives the highest output signal and the highest selectivity at a helium flow rate of between 60 and 90 ml/min. Therefore, in order to obtain both sufficient plates and optimum detector performance, "make up" helium is added to the detector at the column outlet. The nitrogen output signal is almost linear with a carrier gas flow between 60 and 90 ml/min. To ensure a ± 1% signal output stability the carrier gas flow should be kept constant within 0.4%. Detector block temperature is also very important. Higher detector block temperature will increase the selectivity of the detector, due to a larger amount of salt evaporating. Optimum temperature is about 400° C (*N.B.* More detailed information about operating mechanism, reaction mechanism and operation parameters for an A.F.I.D. of the type used by us can be found in HEWLETT-PACKARD (1973) *Operating note* High Sensitivity Nitrogen Detector Model 15161-B, and MAIER-BODE and RIEDMANN, 1975).

In practice the A.F.I.D. proved to be sufficiently sensitive to detect very low amounts of thiazinamium iodide. The detection limit for the pure substance dissolved in absolute ethanol was about 250 pg (giving a peak that was 4 times baseline noise), when operating under optimum circumstances.

We found, however, that there was some variation in detector response. GOUGH and SUGDEN (1973) studied the stability of this type of detector and concluded that the variations in response of the nitrogen detector were higher than those of a normal flame detector. For this reason and to compensate for variations in the injection of the sample we decided to use

a standard for the quantitative work, to correct for the influence of these factors.
This standard would act as an internal standard for the gas chromatographic procedure, but is an external standard for the whole procedure, because it is added after the extraction is performed.
To get such a standard we searched for a phenothiazine derivative with a structure related to promethazine. The retention time of the standard should preferably be a little longer than that of promethazine because co-extracted plasma components may cause some solvent peak tailing before the promethazine peak. Chlorpromazine (retention time about 1.8 times that of promethazine) proved to fulfil these requirements. The standard is injected as chlorpromazine hydrochloride and detected as the amine.

To sum up, we found it possible to isolate thiazinamium methylsulphate from water and to determine nanogram amounts of this drug by employing a combination of ion pair extraction and gas chromatography with alkali flame ionization detector.

We will now go on to describe the procedures of determining thiazinamium methylsulphate in biological fluids.

2.2. Materials

The following materials were used for the determination.

Apparatus
All *gas chromatographic measurements* were performed on a H.P. 5750 Research Gas Chromatograph, equipped with a H.P. High Sensitivity (rubidium bromide) Nitrogen Detector model 15161-B, and connected with a H.P. recorder model 7123-B and a H.P. integrator model 3373-B with baseline correction (Hewlett Packard, Avondale, Pa., U.S.A.).
An all glass system with graphite ferrule connections was used, column length 180 cm, internal diameter 2 mm. Stationary phase 3% S.P. 2100 (Supelco, Inc., Bellafonte, Pa., U.S.A.) on Chromosorb G.-(A.W.-D.M.C.S.)-High Performance 80–100 mesh (Johns-Manville). Injection port temperature: 300° C. Column temperature: 240° C. Detector temperature: 400° C. Carrier gas, helium, flow rate 50 ml/min. Detector gasses: air 180 ml/min., hydrogen 30 ml/min. As additional make up gas helium, 30 ml/min. was added in the detector.

The *pH measurements* were carried out with a Philips digital pH-meter P.W. 9408 (Philips, Eindhoven, The Netherlands).

All *extractions* were done on a Vortex® mixer at maximum velocity.

Centrifugation was done with a Heraeus Christ U.J. II centrifuge.

Glassware was cleaned by standing overnight in chromic acid and then rinsed with distilled water. Phosphate detergents were avoided because of the high sensitivity of the detector for phosphorus compounds.

Reagents
Thiazinamium methylsulphate (3554 R.P.) and chlorpromazine hydrochloride (4560 R.P.) were obtained from SPECIA, Rhône Poulenc, Paris, France. Potassium iodide Suprapur® and ethanol (100%) Pro Analysi® were used without purification; 1,2-dichloroethane Uvasol® (spectrophotometric grade) was purified by distillation in glass before use (E. Merck, Darmstadt, G.F.R.). Heparin solution contained 5 mg (= 500 U) per ml in distilled water. Silyl-8® G.L.C. column conditioner was obtained from Pierce Chemical Company (Rockford, Ill., U.S.A.).

2.3. Determination of thiazinamium cations in plasma

2.3.1. Introduction

The method that was outlined in Section 2.1. will now be discussed in more detail and the choice of various parameters will be explained here.

2.3.2. Methods

Blood samples, of approximately 10 ml were obtained by venous puncture (cubital vein) and collected in glass tubes containing 1 drop of heparin solution (see also Part III, Chapter 2.). After centrifugation 4.0 ml plasma was removed by means of a pipette and put in a centrifuge tube of 50 ml capacity with a Quickfit® stopper.

To the plasma 0.5 ml of a 1.0 molar potassium iodide solution was added. The mixture was shaken on a Vortex® mixer during 10 sec. at maximum velocity. Then 20.00 ml of 1,2-dichloroethane was added and the plasma was extracted under vigorous shaking during 30 sec. on the mixer. The phases were separated by centrifugation during 20 min. at 6000 *g*. The plasma layer and the interphase were aspirated by means of a Pasteur pipette. Of the remaining 1,2-dichloroethane layer 15.00 ml was transferred to a conical glass tube and evaporated in a water bath at 70° C under a gentle stream of nitrogen. The residue was dissolved into 150 µl of ethanol (100%) containing chlorpromazine.HCl (2.5 ng/µl) as a gas chromatographic standard. Of the ethanolic solution an aliquot of 10 µl was injected in the gas chromatograph (for low concentration ranges of thiazinamium a chlorpromazine.HCl solution of 1.25 ng/µl was used and 20 µl was injected into the gas chromatograph.).

2.3.3. Results and discussion

The extraction

A sample size of 4.0 ml plasma appeared to be sufficient for measurement of the plasma concentrations even down to the lower nanogram range (*e.g.* after oral administration of the drug).

As anticoagulants disodium citrate and heparine were tested. Disodium citrate was not useful because of coextraction of several plasma components which may be due to ion pair formation of these components with citrate. In contrast heparin addition did not lead to any disturbance.

The plasma samples were frozen immediately and stored at −20° C until analysis was carried out, which was always within 2 months. No degradation (no decrease in concentration) was found during this period.

As discussed in the introduction, iodide was chosen as the counter ion. For extraction of thiazinamium as iodide ion pair from water chloroform, dichloromethane and 1,2-dichloroethane can be used. For extraction of plasma chloroform appeared to be unfavourable because it led to formation of emulsions. Dichloromethane and 1,2-dichloroethane, however, did not lead to this kind of difficulties. As reported in the introduction dichloromethane gave the best extraction constant for isolation from water in the concentration range of 10^{-4} mol thiazinamium methylsulphate per liter (log E = 2.46, see Table 1). Yet, when extracting plasma with dichloromethane it was found that several other plasma components were also extracted with this solvent, which caused several peaks in the gas chromatogram. In the concentration range just mentioned, the extraction constant for 1,2-dichloroethane was observed to be: log E = 2.09. It was also found that lowering the concentration of the cations leads to an

increase of the conditional extraction constant, probably due to the calculated in the introduction, log $E^* = 3.1$ for the extraction of concentrations of thiazinamium cations from water in concentrations comparable to therapeutic values in plasma. When we assayed the extraction of thiazinamium from plasma with,iodide as a counter ion and 1,2-dichloroethane as extraction solvent, this combination proved to be considerably more selective than the combination of iodide and dichloromethane. The extracts obtained were pure and no interfering peaks were registered with alkali flame ionization detection.

It should be mentioned that it is not feasible to describe and calculate the influence of the phenomenon of dissociation in the organic phase for extractions from a complex material like plasma.

But we found that, under suitable conditions, one extraction with an equal amount of 1,2-dichloroethane was sufficient for an almost quantitative isolation of thiazinamium from plasma (for details such as recovery, time of extraction, amount of counter ion *etc.* see below).

It was also found - as expected for a quaternary ammonium ion - that the efficiency of the extraction was not influenced by the pH of the plasma. It was empirically established that a pH of 10 in general gave minimal co-extraction of interfering compounds, but in practice also extraction at the physiological pH (= 7.4) proved to be usable.

The optimum time of extraction proved to be 30 sec. at maximum velocity. Shorter periods gave incomplete extraction, longer periods too many interfering peaks. After extraction the phases were separated by centrifugation. Application of 6000 *g* for 20 min. was necessary to break the emulsion which is formed during the extraction. In between the aqueous and the organic layer there usually appeared a small interphase of approximately 2 mm. This layer probably exists of coagulated peptides. It must be aspirated completely together with the aqueous layer, because it can cause tailing of the solvent peak in the chromatographic experiments.

After the aspiration of the two top layers 15.00 ml of the organic layer was transferred to a conical glass tube for a concentration step. As discussed in the introduction, halogenated solvents should not be injected in a gas chromatographic system with an alkali flame ionization detector that contains a rubidium bromide crystal. Hence we decided to evaporate the 1,2-dichloroethane completely and to dissolve the residue in a solvent not containing halogen atoms. To avoid oxidation of the sample the evaporation was done under a gentle stream of nitrogen. Because 1,2-dichloroethane has a rather high boiling point (83–84° C) warming was necessary to complete the procedure in a reasonable time. On the other hand, oxidation of thiazinamium will be favoured by raising the temperature. We found that evaporation in a water bath at 70° C under nitrogen could be adequately completed in 30 min. without causing oxidation of thiazinamium.

For re-uptake of thiazinamium iodide in the dry residue, methanol, ethanol

TABLE 3: *Solubility of the ion pair thiazinamium iodide in various solvents according to the procedure described in Part I, 2.6.*

Solvent	Solubility mg/ml	Solubility mol/l
water (pH = 5.9)	2.9	6.8×10^{-3}
methanol (100%)	192.0	4.5×10^{-1}
ethanol (100%)	35.8	8.4×10^{-2}
1-propanol (100%)	14.3	3.4×10^{-2}

and 1-propanol were tested in varying amounts. Although thiazinamium iodide rapidly dissolved in small amounts (50 µl) of methanol, the latter also dissolved various interfering components, which caused unwanted extra peaks in the gas chromatogram. Ethanol 100% proved more suitable: a minimum amount of other components was co-extracted in this solvent and they did not interfere with thiazinamium iodide. As a matter of fact, the solubility of the ion pair in ethanol is less than in methanol, so the volume of ethanol required is somewhat higher (150 µl). The solubility in 1-propanol was too low to allow its use as solvent (see Table 3). For complete dissolution of the dry residue it was necessary to shake the tube vigorously on a Vortex® mixer for appr. 20 sec. and the total glass surface had to be moistened with the ethanol.
We also investigated dissolution under ultrasonic vibration. This shortened dissolution time, but increased the amount of interfering components in the final solution.

The gas chromatographic procedure
With the gas chromatographic procedure described under "Apparatus" the retention time of promethazine (the demethylation product of thiazinamium iodide) was appr. 350 sec. and that of chlorpromazine (standard) about 630 sec. (Fig. 3.). The several preconditions which should be taken to avoid degradation and adsorption on the column have already been discussed in detail in the "Introduction". Each day, one hour before use, the column system was silanized by 5 injections of 10 µl of Silyl-8® G.L.C. column conditioner. This procedure was repeated every 4 hours of use because the Silyl-8® was washed away from the column at the high oven temperature. During this procedure the column was disconnected from the detector to avoid silylation of the rubidium bromide crystal, which otherwise would cause decrease of sensitivity.

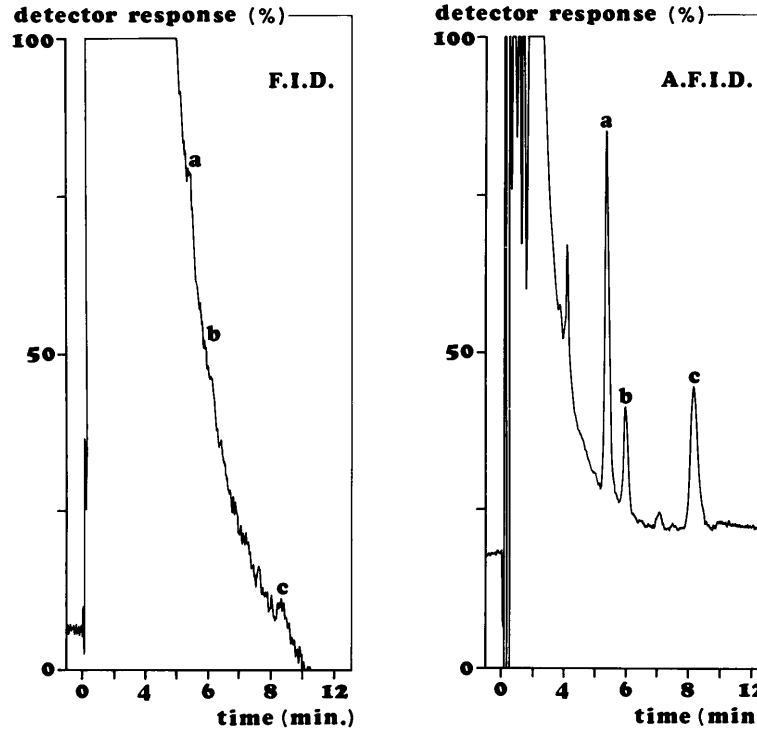


FIG. 3: *Gas chromatograms of a plasma extract.*
Left: Normal Flame Ionization Detector (F.I.D.). Range: 10. Attenuation: 4.
Right: Alkali Flame Ionization Dectector (A.F.I.D.). Range: 100. Attenuation: 4.
a = promethazine formed by demethylation of thiazinamium iodide (= 47.7 ng).
b = di-(2-ethylhexyl)phtalate, contaminant from the PVC cap liner of the bottle of the extraction solvent, probably coinciding with an unknown compound extracted from plasma.
c = gas chromatographic standard chlorpromazine (25 ng).

As described above, the optimal use of an A.F.I.D. requires exact adjustment of several parameters. The flow rates of the gasses after having been adjusted to give optimal response, should be checked regularly. The distance between the rubidium bromide crystal and the top of the burner jet is of major importance and must be adjusted with precision. This was done daily by monitoring the detector response of a 1 µl injection of a mixture of 500 mg of octadecane and 1 mg of azobenzene per 100 ml of n-hexane. Suitable conditions by correct adjustment have been achieved when the response of the azobenzene is several times (*e.g.* 5 times) that of the octadecane. Before use the surface of the crystal has to be cleaned with a special brush (at regular intervals, *e.g.* each day).The sensitivity decreases

strongly if the surface of the crystal is dirty. During the gas chromatographic procedure a white coating appeared on the surface of the crystal. This is probably SiO_2 deposited due to degradation of the silicone rubber stationary phase and of the Silyl-8®. After prolonged use (*e.g.* one month) the surface of the crystal would become concave as a consequence of evaporation of the part of the crystal just above the top of the flame. The surface can be made smooth and flat again with the help of very fine sandpaper.
For quantitative work a calibration curve of 5-100 ng of thiazinamium iodide versus 25 ng of chlorpromazine.HCl (both dissolved in ethanol 100%) was used (Fig. 4.). The amounts of thiazinamium iodide measured were converted to the corresponding amount of the methylsulphate.

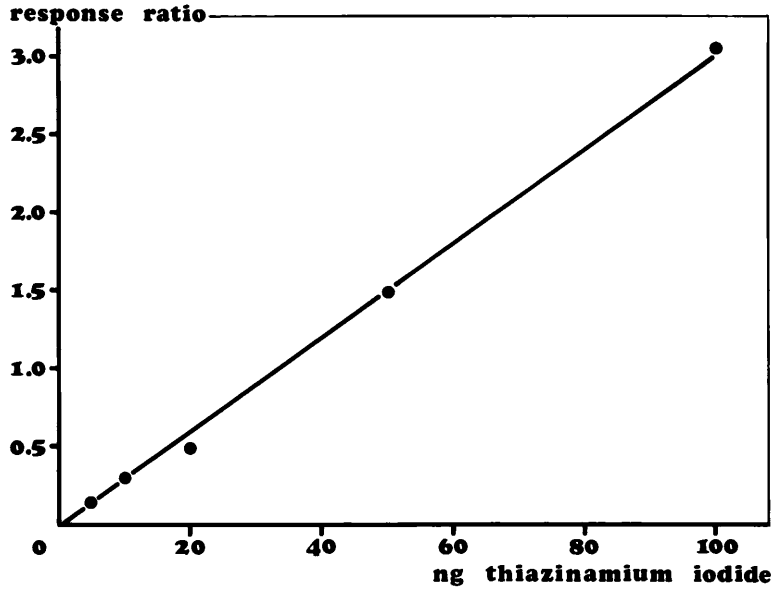


FIG. 4: *Calibration graph for the determination of thiazinamium cations - as iodide ion pair - in plasma, using 25 ng chlorpromazine.HCl as a standard. The response ratio represents the ratio of the peak area of thiazinamium iodide and that of chlorpromazine.HCl, both injected as the pure substance into the gas chromatograph.*

Selectivity, sensitivity, recovery and reproducibility of the total procedure
When normal human plasma was examined as described no blank values were observed. The only metabolite we could find in man, thiazinamium sulphoxide, is much more polar than the parent drug and is not extracted in measurable amounts from the plasma or urine in this way (see Chapter 3). Other drugs, except promethazine itself, are unlikely to interfere because of the selectivity of the combined procedure. Therefore patients had to be

selected which were hold free of promethazine for at least two weeks.
During the early stages of our investigations it was found that the peaks caused by thiazinamium iodide and chlorpromazine. HCl were accompanied by a third peak with a retention time of approximately 450 sec. This peak could be identified by means of mass spectrometry and U.V., I.R. and N.M.R. spectra as di-(2-ethylhexyl)phtalate, a plasticiser, often used in the manufacture of plastics of the polyvinylchloride type. The source of contamination was identified as the cap liners of the high purity 1,2-dichloroethane. The component was removed for the greater part by distillation (DE ZEEUW, JONKMAN & others, 1975). Often the peak on this place in the extract was larger than was found in the pure solvent which suggests that this phtalate or a compound with the same gas chromatographic properties was coextracted from the plasma.
The sensitivity of the total procedure was such that plasma concentrations of 2 ng/ml (= 4.87 picomol/ml) could be detected and accurate quantitation could be done at concentrations of 20 ng/ml (= 48.7 picomol/ml) and above. When the concentration was expected to be below approximately 40 ng/ml plasma, the residue was dissolved in 150 µl of a solution of 1.25 ng chlorpromazine.HCl/µl (instead of 2.50 ng/µl) and 20 µl (instead of 10 µl) were injected into the gas chromatograph.
As discussed in Part II, Chapter 1, the extraction constant of an ion pair extraction is influenced by the amount of counter ion added. We investigated the influence of the amount of iodide on the recovery from water which was spiked with a low concentration of thiazinamium cations, in order to calculate the minimum amount of iodide required. Thiazinamium methylsulphate was dissolved in water so that a concentration of 320.0 ng/ml (= 7.80×10^{-7} mol/l) was obtained. This concentration represents therapeutic concentrations in plasma. Different amounts of potassium iodide were added in such a way that the initial concentrations in the aqueous phase varied from 5.0×10^{-6} to 2.0×10^{-1} mol/l. For each concentration four extractions were carried out and the mean value and standard deviation from the mean were calculated. The results are shown in Fig. 5, which indicate that the recovery does not increase anymore at a concentration of 5.0×10^{-3} mol/l and above. Theoretically the percentage recovery of thiazinamium cations from an aqueous solution can be calculated using equation VIII (Part II, Chapter 1; see also SCHILL, 1974). For a concentration of thiazinamium of approximately 5×10^{-7} mol/l a recovery of 99% (which would be very satisfactory), corresponding with a D = 27.2 for the described procedure, would be obtained with a $c'_{\bar{1}aq} = 1.86 \times 10^{-2}$ mol/l.
In practice, at this concentration of iodide ions the recovery was between 80 and 90% (Fig. 5). Higher concentrations of iodide ion gave no further increase of the recovery, probably because of adsorption on glass and/or accumulation at the interface between the aqueous and the organic layer.

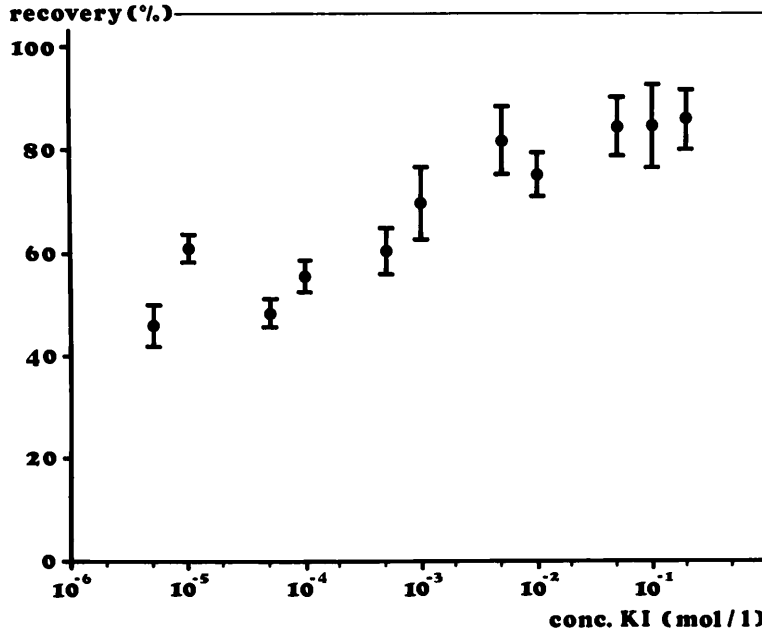


FIG. 5: *Recovery of the extraction of thiazinamium – extracted as iodide ion pair – from water with 1,2-dichloroethane using various initial concentrations of the iodide counter ion. Concentration of thiazinamium methylsulphate is 320.0 ng/ml (= 7.80 × 10^{-7} mol/l), pH = 10. Each point represents the mean value of four determinations with standard deviations in brackets.*

Although for optimum extraction from water the minimum initial concentration of I^- counter ions thus appeared to be appr. 5.0 × 10^{-3} mol/l, it was found that a quantitative extraction from a complex sample like plasma requires a higher initial iodide concentration. This is probably due to additional side reactions, such as protein binding of the iodide or coextraction of other iodide ion pairs. WESTERLUND and KARSET (1973) reported similar findings in the extraction of propantheline with perchlorate from plasma.

In practice an iodide concentration of 1.0 × 10^{-1} mol/l appeared to be sufficient to achieve an adequate recovery.

The recovery of the total procedure for the extraction of thiazinamium cations from plasma was determined for six different concentrations. Plasma of a volunteer was divided into six portions. Each portion was spiked with thiazinamium methylsulphate in such a way that the concentrations covered the range in which the therapeutic levels were found after several routes of administration of the drug to patients. Recoveries were determined in sevenfold in each portion and the mean value and the standard deviation from the mean were calculated. The values obtained are

TABLE 4: *Recovery of thiazinamium cations – extracted as iodide ion pair - from plasma. The initial concentration of iodide is 1.0 × 10^{-1} mol/l.*

initial concentration of thiazinamium methylsulphate		recovery mean ± S.D. %	number of determinations
ng/ml	mol/l		
40.0	9.75×10^{-8}	93.4 ± 8.8	7
80.0	1.95×10^{-7}	87.0 ± 8.3	7
160.0	3.90×10^{-7}	86.3 ± 4.0	7
240.0	5.85×10^{-7}	91.9 ± 6.5	7
320.0	7.80×10^{-7}	84.5 ± 4.9	7
480.0	1.70×10^{-6}	90.5 ± 8.1	7

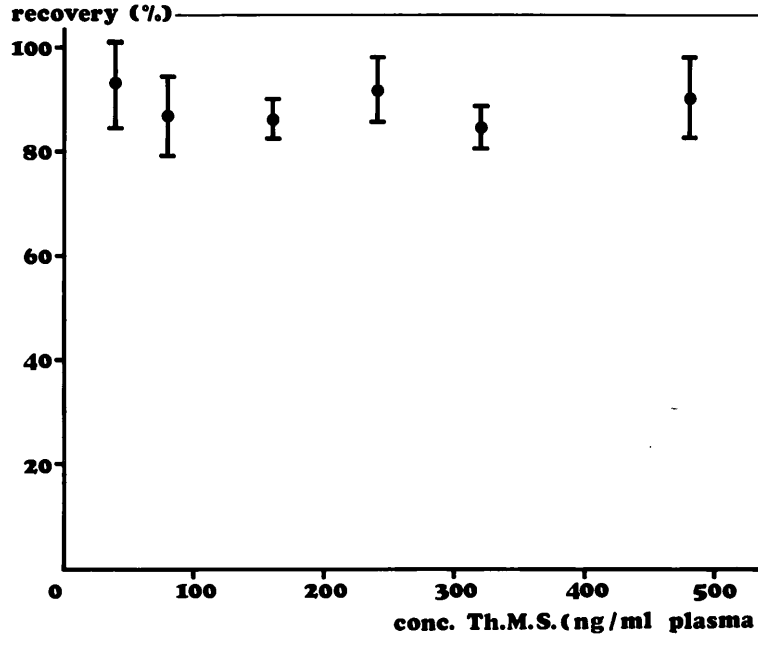


FIG. 6: *Recovery of the extraction of thiazinamium cations - extracted as iodide ion pair - from plasma at different initial concentrations of thiazinamium methylsulphate (= Th. M. S.). The initial concentration of iodide is 1.0 × 10^{-1} mol/l.*

given in Table 4. The results are graphically represented in Fig. 6. As Table 4 shows, the standard deviation was not correlated with the concentration. We therefore determined an overall mean recovery with standard deviation for the whole therapeutic range of plasma concentrations. A mean value of 88.6 ± 7.9 (S.D.) % was found (n = 42). In the determination of concentrations of thiazinamium methylsulphate in plasma a recovery of 88.6% was taken into account and all values obtained were corrected for it to 100% recovery.

Conclusion

The method described for the determination of thiazinamium methylsulphate in plasma is relatively rapid and selective, gives a good recovery and is sufficiently reproducible. The metabolite does not interfere with determination of the drug. Its sensitivity allows determination of plasma concentrations as they are obtained after administration of therapeutic doses of the drug. For these reasons the method is suitable for pharmacokinetic studies.

2.4. Determination of thiazinamium cations in urine

2.4.1. Introduction

An essential difference between urine and plasma analysis is the concentration of the drug in these two body fluids. In plasma, concentrations in the nanogram range were found, but in urine, although strongly dependent on the dose and route of administration, concentrations were in the microgram range. Thus smaller samples could be used, but otherwise it was found that the procedure as described for plasma was equally suitable for urine, only minor modifications being required.

2.4.2. Methods

The volume of the urine sample was measured and 0.5 ml was transferred to a centrifuge tube of 50 ml capacity with a Quickfit® stopper. The sample was diluted with 1.5 ml of distilled water and 0.5 ml of a 1 molar potassium iodide solution was added. The mixture was then shaken during 10 sec. on a Vortex® mixer at maximum velocity. Next 20.00 ml of 1,2-dichloroethane was added and the plasma was extracted under vigorous shaking during 30 sec. on the mixer. The phases were separated by centrifugating during 20 min. at 6000 *g*. The aqueous layer was aspirated by means of a Pasteur pipette. Of the remaining 1,2-dichloroethane layer 15.00 ml was transferred to a conical glass tube and the 1,2-dichloroethane was then evaporated in a water bath at 70° C under a gentle stream of nitrogen. The residue was dissolved in 150 µl of ethanol (100%) containing chlorpromazine.HCl (25 ng/µl) as a gas chromatographic standard. Of the ethanolic solution an aliquot of 10 µl was injected in the gas chromatograph.

2.4.3. Results and discussion

A sample size of 0.5 ml urine proved to be sufficient for measurements of thiazinamium in urine. This quantity was taken from urine samples that were frozen directly after the clinical experiment and stored at − 20° C until analysis, which generally took place within 2 months. No degradation (no decrease in the concentration) was found during this period. Intermediate thawing and refreezing (sometimes necessary for determination of thiazinamium sulphoxide) did not influence the amount of thiazinamium in the samples either.
The samples were diluted in order to facilitate separation of the phases after extraction and centrifugation.
The concentration of iodide was of the same order of magnitude as in the case of the determination in plasma.
In general the pH of the urine samples varied from 5 to 8. In the early stages of our investigations the pH of each sample was measured and subsequently adjusted to 7. After it had been found that recovery was independent of pH this step was omitted.

Selectivity, sensitivity, recovery and reproducibility of the procedure
When normal human urine was examined no blank values were observed. Although the concentration of the metabolite thiazinamium sulphoxide was much higher here than in plasma, especially after oral and rectal administration of the drug, it did not disturb the analysis.

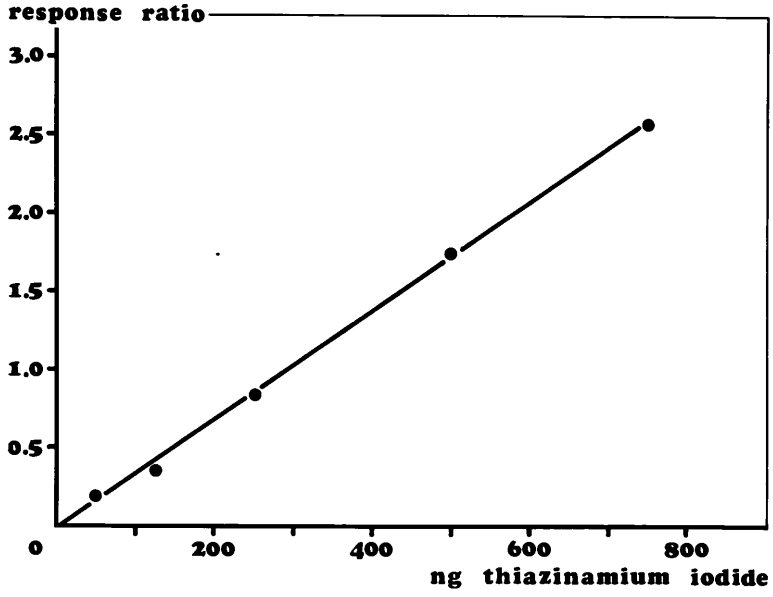


FIG. 7: *Calibration graph for the determination of thiazinamium cations - as iodide ion pair - in urine, using 250 ng chlorpromazine.HCl as a standard. The response ratio represents the ratio of the peak area of thiazinamium iodide and chlorpromazine.HCl, both injected as the pure substance.*

Because of these higher concentrations of thiazinamium in urine in comparison with plasma the gas chromatograph was set at a higher "attenuation". A calibration curve was used with concentrations ranging from 50–750 ng of thiazinamium iodide against 250 ng of chlorpromazine.HCl (see Fig. 7).

The amount of chlorpromazine.HCl added to the residue was such that 10 µl of the final solution also contained 250 ng of this standard. Although in principle the sensitivity of the procedure was the same as for plasma (using the calibration curve against 25 ng of chlorpromazine.HCl) it was not necessary to work at maximum sensitivity. In some cases the concentrations were so high that the values for the response ratio were not covered by the calibration curve. Then the ethanolic solution was diluted with the chlorpromazine.HCl solution until reading was possible.

The recovery of the total procedure for the analysis of thiazinamium cations in urine was determined for five different concentrations. Urine from a volunteer was divided into five portions. Each portion was spiked with an aqueous thiazinamium methylsulphate solution in such a way that concentrations were obtained which are of the same order of magnitude as those generally found in urine after administration of the drug. Recoveries were determined in sevenfold in each portion and the mean value and

TABLE 5: *Recovery of thiazinamium cations - extracted as iodide ion pair - from urine. The initial concentration of iodide is 2.0 × 10^{-1} mol/l.*

initial concentration of thiazinamium methylsulphate		recovery mean ± S.D. %	number of determinations
µg/ml	mol/l		
5.0	1.22 × 10^{-5}	88.8 ± 8.1	7
7.0	1.71 × 10^{-5}	91.4 ± 10.2	7
10.0	2.44 × 10^{-5}	98.7 ± 6.6	7
20.0	4.87 × 10^{-5}	86.7 ± 3.1	7
40.0	9.74 × 10^{-5}	89.6 ± 7.8	7

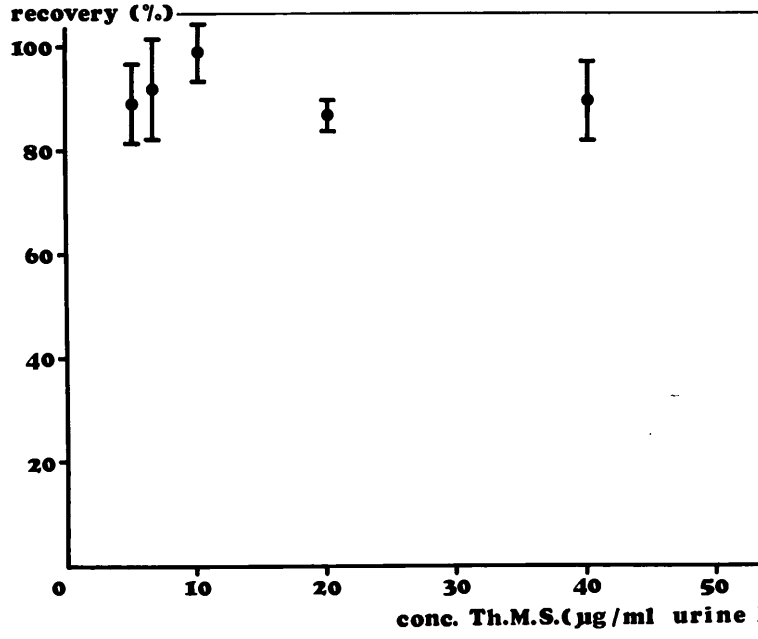


FIG. 8: *Recovery of the extraction of thiazinamium cations - extracted as iodide ion pair - from urine at different initial concentrations of thiazinamium methylsulphate (= Th.M.S.).*
The initial concentration of iodide is 2 × 10^{-1} mol/l.

standard deviation from the mean were calculated. The values obtained are given in Table 5.
The results are graphically represented in Fig. 8.
When all values for the recovery were pooled and the mean value and standard deviation were calculated a value of 90.99% ± 8.13 (S.D.) was obtained (n = 35). As can be seen from Table 5 and Fig. 8 the values obtained for urine are comparable to those obtained with plasma (Table 4, Fig. 6).
In the determination of concentrations of thiazinamium methylsulphate in urine a recovery of 90.99% was taken into account and all values obtained were corrected by conversion to 100% recovery.

Conclusion

The method described for the determination of thiazinamium methylsulphate in urine – just like the method for plasma – is relatively rapid and selective. Recovery is good and reproducible, while the values obtained are of the same order of magnitude as in the case of plasma. The metabolite, although sometimes present in an equal concentration as the parent drug, does not interfere with the determination of thiazinamium cations.
The method is sufficiently sensitive to allow determination of concentrations in urine such as are obtained after administration of therapeutic doses of the drug. The method is suitable therefore for pharmacokinetic studies.

2.5. Determination of thiazinamium cations in bile

2.5.1. Introduction

Because it is well known (SCHANKER and SOLOMON, 1963; MEIJER, BOS and others, 1970; SMITH, 1971) that quaternary ammonium compounds can be excreted in the bile, we decided to investigate whether this is also the case with thiazinamium methylsulphate. To this end a method for the determination of the drug in the bile was required and we investigated to what extent the method as described for plasma and/or urine could be applied to bile.

2.5.2. Methods

The volume of the bile sample, obtained as described in Part III, Chapter 9, was measured and 0.2 ml was homogenized and transferred to a centrifuge tube of 50 ml capacity with a Quickfit® stopper. The sample was diluted with 1.8 ml of distilled water and 0.5 ml of a 1 molar potassium iodide solution was added. The mixture was then shaken during 10 sec. on a Vortex® mixer at maximum velocity. Next 20.00 ml of 1,2-dichloroethane was added and the bile was extracted under vigorous shaking for 30 sec. on the mixer. The phases were separated by centrifugating for 20 min. at 6000 *g*. The bile layer and the interphase were aspirated by means of a Pasteur pipette. Of the remaining 1,2-dichloroethane layer 15.00 ml was transferred to a conical glass tube and evaporated in a water bath at 70° C under a gentle stream of nitrogen.
Depending on the concentration of thiazinamium cations two methods were used for the ensuing procedure.

A. Concentrations lower than 10 µg thiazinamium methylsulphate per ml bile

The residue was dissolved in 150 µl of ethanol (100%) containing chlorpromazine.HCl (2.5 ng/µl) as a gas chromatographic standard. Of the ethanolic solution an aliquot of 10 µl was injected into the gas chromatograph.

B. Concentrations higher than 10 µg thiazinamium methylsulphate per ml bile

The residue was dissolved in 150 µl of ethanol (100%) containing chlorpromazine.HCl (25 ng/µl) as a gas chromatographic standard. Of the ethanolic solution an aliquot of 10 µl was injected into the gas chromatograph.

2.5.3. Results and discussion

The nature of the various bile samples obtained from different patients showed considerable variation, depending on bile production of the patient and collection time. Some samples were the result of a bile production of appr. 5 ml per hour, while in other cases the production was appr. 35 ml per hour. When the sample size was small because of low bile production, the bile was dark in colour, whereas the larger samples were often more diluted and less intensely coloured. Because it appeared that some coloured substances of the bile were extracted by 1,2-dichloroethane, we decided to use only a small part of the total sample, namely 0.2 ml. In this way column

contamination was reduced to a minimum. The concentrations of thiazinamium cations in bile were sufficiently high to allow accurate determination in small volumes. The samples were diluted with water to facilitate separation of the phases after extraction and centrifugation. Application of 6000 *g* for 20 min. was necessary to break the emulsion which is formed during the extraction. In between the aqueous and the organic layer there usually appeared a small interphase. This must be aspirated completely together with the aqueous phase, because it can cause tailing of the solvent peak in the chromatographic experiments.
Because the concentrations of thiazinamium methylsulphate in the 0.2 ml portion of the bile sample may vary appreciably as a result of differences in bile production, two methods were used for dissolving the residue after evaporation of the extraction solvent and for the subsequent gas chromatographic procedure. In cases of diluted slightly coloured bile containing rather low concentrations of thiazinamium cations we use the procedure as described for plasma, with 2.5 ng of chlorpromazine.HCl per µl as a standard and using the calibration curve for plasma extractions. When the concentrations were above appr. 10 µg of thiazinamium methylsulphate per ml bile, the dissolution procedure and subsequent gas chromatographic procedure as described for urine was used, with 25 ng of chlorpromazine.HCl per µl as a standard.

Selectivity, sensitivity, recovery and reproducibility of the procedure
When bile samples were extracted utilizing the above procedure in most instances no blank values were obtained.
The recovery of the two procedures was also tested. Bile, obtained from a patient before administering the drug, was divided in various portions. Each portion was spiked with an aqueous solution of thiazinamium methylsulphate to give a concentration of 5.00 and 18.75 µg/ml respectively, covering the range of concentrations which were found after administration of a therapeutic dose of the drug.
The results are given in Table 6. As can be seen from this table the recovery

TABLE 6: *Recovery of thiazinamium cations - extracted as iodide ion pair - from bile. For methods used see text.*

Method	initial concentration of thiazinamium methylsulphate		recovery mean ± S.D. %	number of determinations
	µg/ml	mol/l		
A	5.00	1.22×10^{-5}	100.4 ± 4.9	8
B	18.75	4.57×10^{-5}	105.6 ± 3.3	4

was about 100% in either case. As this correlated well with the previous obtained results for plasma and urine, for each procedure only one concentration was tested.
In the determination of thiazinamium methylsulphate in bile a recovery of 100% was taken into account.

Conclusion

Bile proved to be a rather complex material that contained more components which are liable to interfere with the analysis of thiazinamium than in the case of plasma or urine. However, the concentrations of thiazinamium cations in the bile proved so high that they allowed small samples to be taken for investigation. Depending on the concentration the procedure followed for plasma or urine could be applied. Recovery was approximately 100%. The method proved to be suitable for pharmacokinetic studies.

2.6. Determination of thiazinamium cations in saliva

2.6.1. Introduction

For several drugs occurrence in saliva has been reported as for example for some antibiotics (BORZELLECA and CHERRICK, 1965), antipyrine (WELCH and others, 1975; FRASER and others, 1976), barbiturates (RASMUSSEN, 1964; BORZELLECA and DOYLE, 1966; INABA and KALOW, 1975), digoxin (HUFFMAN, 1975), lithium (GROTH and others, 1974), paracetamol (GLYNN and BASTAIN, 1973), phenytoin (COOK and others, 1975), salicylic acid (BORZELLECA and DOYLE, *l.c.*; BORZELLECA and PUTNEY, 1970; GRAHAM and ROWLAND, 1972; CHIOU and others, 1976), sulphonamides (RASMUSSEN, *l.c.*; BORZELLECA and DOYLE, *l.c.*), theophylline (KOYSOOKO and others, 1974) and tolbutamide (MATIN and others, 1974).
The process of drug excretion in saliva is not well known. KEEN (1971) suggests that drugs appear in saliva as a result of passive diffusion of the free, non-protein-bound fraction of the drug in plasma, especially of the nonionized moiety. On the other hand active secretion seems to exist too

(RITSCHEL, 1976) as has been reported for *e.g.* lithium (GROTH, *l.c.*). RITSCHEL (1973) stated that no possibility would seem to exist for ionized drugs to be excreted into saliva.
Although no reports are available in the literature about excretion of quaternary ammonium compounds into saliva, we decided to study this route of excretion for thiazinamium methylsulphate for two reasons. Firstly, it is well known from clinical observation that a dry mouth can appear as side effect after administration of thiazinamium methylsulphate, which is an anticholinergic effect. Secondly, our autoradiographic studies (see Part III, Chapter 13) gave indications of rather high amounts of the drug in the salivary glands.
In order to study a possible excretion in saliva, we have investigated which of the methods described in the previous chapters could be applied to saliva.

2.6.2. Methods

A volume of not less than 1.0 ml (but if available up to 4.0 ml) of saliva was transferred to a centrifuge tube of 50 ml capacity with a Quickfit® stopper. The sample was diluted with 2.0 ml of distilled water and the procedure as described for plasma (Part II, Section 2.3.2.) was followed.

2.6.3. Results and discussion

Obviously due to the side effect mentioned above, in general rather small saliva samples are available after administration of thiazinamium methylsulphate. For this reason the most sensitive one of the described methods, namely that for plasma, was selected for testing the determination of thiazinamium cations in saliva. Because saliva may contain much mucus and because interaction between mucus and quaternary ammonium ions is a well known phenomenon, studying of the efficiency of the extraction was required.

Selectivity, sensitivity, recovery and reproducibility of the procedure
When normal saliva samples were analyzed utilizing the above procedure in most instances no blank values were obtained.
Because rather clean extracts were obtained almost no interference from the solvent peak appeared, which allowed injections of 20 µl of the final ethanol solution. So, the sensitivity was at least as high as reported in the method for plasma, but due to the small volumes of saliva which were generally available (appr. 1 ml) the detection limit is now appr. 8 ng/ml whereas

TABLE 7: *Recovery of thiazinamium cations - extracted as iodide ion pair - from saliva.*

initial concentration of thiazinamium methylsulphate		recovery mean ± S.D. %	number of determinations
ng/ml	mol/l		
80.0	1.95×10^{-7}	87.4 ± 8.3	7
160.0	3.90×10^{-7}	92.9 ± 7.1	7

accurate quantitation can be done from 50 ng/ml and up.
The recovery of the procedure was determined for two different concentrations. Saliva, obtained from patients before giving the drug, was divided in two portions. To the saliva an aqueous solution of thiazinamium methylsulphate was added in such a way that concentrations of 80 and 160 ng/ml were obtained respectively. The results are given in Table 7. As can be seen from this table the recovery was approximately the same as for plasma, which means that little or no irreversible binding to mucus occurs. Also the relative standard deviations were almost the same as were found for analysis in plasma.

Conclusion

Saliva gave in general rather clean extracts which allowed determination of low concentrations of thiazinamium methylsulphate. Recovery was found to be approximately the same as for the determination of the drug in plasma.

References

AHUJA, S. (1976), *J. Pharm. Sci. 65,* 163

ALLGÉN, L. G., L. EKMAN, L. REIO and S. ULLBERG (1960), *Arch. int. Pharmacodyn. Thér. 126,* 1

AUE, W. A., G. W. GEHRKE, R. C. TINDLE, D. L. STALLING and D. D. RUYLE (1967), *J. Gas Chromatog. 5,* 381

BERTI, T. and L. CIMA (1954), *Arch. int. Pharmacodyn. Thér. 98,* 452

BILZER, W. and U. GUNDERT-REMY (1973), *Europ. J. Clin. Pharmacol. 6,* 268

BOON, P. F. G. and A. W. MACE (1969), *J. Chromatog. 41,* 105

BORG, K. O. (1969), *Acta Pharm. Suecica 6,* 425

BORZELLECA, J. F. and H. M. CHERRICK (1965), *J. Oral Ther. Pharmacol. 2,* 180

BORZELLECA, J. F. and C. H. DOYLE (1966), *J. Oral Ther. Pharmacol. 3,* 104

BORZELLECA, J. F. and J. W. PUTNEY (1970), *J. Pharmacol. Exp. Ther. 174,* 527

BREIMER, D. D. (1974), in: *"Pharmacokinetics of Hypnotic Drugs"* (thesis), Drukkerij Brakkenstein, Nijmegen, The Netherlands
BREIMER, D. D. and J. M. VAN ROSSUM (1974), *J. Chromatog. 88,* 235
CHIOU, W. L., K. CHANG and G. W. PENG (1976), *J. Clin. Pharmacol. 16,* 158
CIMBURA, G. (1972), *J. Chromatog. Sci. 10,* 287
COOK, C. E., E. AMERSON, W. K. POOLE, P. LESSER and L. OTUAMA (1975), *Clin. Pharmacol. Ther. 18,* 742
CREMER, E. (1967), *J. Gas Chromatog. 5,* 329
CURRY, S. H. (1968), *Anal. Chem. 40,* 1251
CURRY, S. H. (1974), in: *"The Phenothiazines and Structurally Related Drugs"* (Eds.: I. S. Forrest, C. J. Car and E. Usdin), Raven Press, New York
CURRY, S. H. and B. B. BRODIE (1967), *Fed. Proc. 26,* 761
CURRY, S. H. and G. P. MOULD (1969), *J. Pharm. Pharmacol. 21,* 674
DE LEENHEER, A. (1973), *J. Chromatog. 77,* 339
DE LEENHEER, A. P. (1974), *J. Pharm. Sci. 63,* 389
DE ZEEUW, R. A., J. H. G. JONKMAN and F. J. W. VAN MANSVELT (1975), *Anal. Biochem. 67,* 339
DONIKE, M., J. JAENICKE, D. STRATMANN and W. HOLLMANN (1970), *J. Chromatog. 52,* 237
DRISCOLL, J. L., H. F. MARTIN and B. J. GUDZINOWICZ (1964), *J. Gas Chromatog. 2,* 109
FLINT, D. R., C. R. FERULLO, P. LEVANDOSKI and B. HWANG (1971), *Clin. Chem. 17,* 830
FRASER, H. S., J. C. MUCKLOW, S. MURRAY and D. S. DAVIES (1976), *Brit. J. Clin. Pharmacol. 3,* 321–325
GLYNN, J. P. and W. BASTAIN (1973), *J. Pharm. Pharmacol. 25,* 420
GOUDIE, J. H. and D. BURNETT (1973), *Clin. Chim. Acta 43,* 423
GOUGH, T. A. and K. SUGDEN (1973), *J. Chromatog. 86,* 65
GRAHAM, G. and M. ROWLAND (1972), *J. Pharm. Sci. 61,* 1219
GROTH, U., W. PRELLWITZ and E. JÄHNCHEN (1974), *Clin. Pharmacol. Ther. 16,* 490
GUDZINOWICZ, B. J., H. F. MARTIN and J. L. DRISCOLL (1965), *J. Gas Chromatog. 3,* 265
HAMMAR, C. G. and B. HOLMSTEDT (1968), *Experientia 24,* 98
HAMMAR, C. G., B. HOLMSTEDT and R. RYHAGE (1968), *Anal. Biochem. 25,* 532
HARTIGAN, M. J., J. E. PURCELL, M. NOVOTNY, M. L. MCCONNELL and M. L. LEE (1974), *J. Chromatog. 99,* 339
HEWLETT-PACKARD (1973), *Operating note* High Sensitivity Nitrogen Detector Model 15161-B
HUCKER, H. B. and S. C. STAUFFER (1976), *J. Pharm. Sci. 65,* 926
HUFFMAN, D. H. (1975), *Clin. Pharmacol. Ther. 17,* 310
INABA, T. and W. KALOW (1975), *Clin. Pharmacol. Ther. 18,* 558
JACKSON, J. V. (1971), in: *"Isolation and Identification of Drugs"*, E. G. C. Clarke, Ed., The Pharmaceutical Press, London
JAIN, N. C. and P. L. KIRK (1967), *Microchem. J. 12,* 256
JAMES, S. P. and R. H. WARING (1971), *J. Chromatog. 78,* 417
JANAK, J., V. SVOJANOVSKY and M. DRESSLER (1968), *Czech. Chem. Commun. 33,* 740
JENDEN, D. J., I. HANIN and S. J. LAMB (1968), *Anal. Chem. 40,* 125
JENNINGS, E. C. and H. MITCHNER (1967), *J. Pharm. Sci. 56,* 1590
JOHNSON, D. E., C. F. RODRIGUEZ and W. SCHLAMEUS (1965), *J. Gas Chromatog. 3,* 345
KARMEN, A. (1964), *Anal. Chem. 36,* 1416
KARMEN, A. and L. GIUFFRIDA (1964), *Nature 201,* 1204

KEEN, P. (1971), "Effect of binding to plasma proteins on the distribution, activity and elimination of drugs" in *Concepts in Biochemical Pharmacology*, B. B. Brodie, J. R. Gilette, Eds., Springer Verlag, Berlin, Heidelberg, New York
KELSEY, M. I. and E. A. MOSCATELLI (1973), *J. Chromatog. 85*, 65
KERCKHOFFS, H. P. M. and T. HUIZINGA (1967), *Pharm. Weekblad 102*, 1183
KLEINSORGE, H., K. THALMAN and K. RÖSNER (1959), *Arzneimittel-Forsch. 9*, 121
KOFOED, J., C. KORCZAK-FABIERKIEWICZ and G. H. W. LUCAS (1966), *Nature (London) 211*, 147
KOLB, B. and J. BISCHOFF (1974), *J. Chromatog. Sci. 12*, 625
KOYSOOKO, R., E. F. ELLIS and G. LEVY (1974), *Clin. Pharmacol. Ther. 15*, 454
LARSEN, N. E. and J. NAESTOFT (1975), *J. Chromatog. 109*, 259
MAIER-BODE, H. and M. RIEDMANN (1975), in: *Residue Reviews*, Vol. 54, F. A. Gunther and J. Davies Gunther, Eds., Springer Verlag, New York, Berlin
MARTIN, H. F., J. L. DRISCOLL and B. J. GUDZINOWICZ (1963), *Anal. Chem. 35*, 1901
MATIN, S. B., S. H. WAN and J. H. KARAM (1974), *Clin. Pharmacol. Ther. 16*, 1052
MEFFIN, P. J., G. MOORE and J. THOMAS (1973), *Anal. Chem. 45*, 1964
MEYER, D. K. F., E. S. BOS and K. J. VAN DER LAAN (1970), *Europ. J. Pharmacol. 11*, 371
MITCHNER, H. and E. C. JENNINGS (1967), *J. Pharm. Sci. 56*, 1595
MODIN, R. and G. SCHILL (1970), *Acta Pharm. Suecica 7*, 585
MULTERGAN® 3554 R. P. (1961), *Note technique*, SPECIA, Rhône Poulenc, Paris, France
NATUSH, D. F. S. and T. M. THORPE (1973), *Anal. Chem. 45*, 1184A
RASMUSSEN, F. (1964), *Acta Pharmacol. Toxicol. 21*, 11
RIEDMANN, M. (1972a), *Naturwissenschaften 59*, 306
RIEDMAN, M. (1972b), *Chem. Zeitung, Sonderdruck 96*, 618
RIEDMANN, M. (1973), *Xenobiotica 3*, 411
RIEDMANN, M. (1974a), *J. Chromatog. 88*, 376
RIEDMANN, M. (1974b), *J. Chromatog. 92*, 55
RITSCHEL, W. A. (1973), in: *"Angewandte Biopharmazie"*, Wissenschaftliche Verlagsgesellschaft, Mbh. Stuttgart
RITSCHEL, W. A. (1976), in: *"Handbook of Basic Pharmacokinetics"*, Drug Intelligence Publications, Inc., Hamilton, Il.
RIVERA-CALIMLIM, L., L. CASTANADA and L. LASAGNA (1973), *Clin. Pharmacol. Ther. 14*, 978
SCHANKER, L. S. and H. M. SOLOMON (1963), *Am. J. Physiol. 204*, 829
SCHILL, G. (1974), in: *"Ion Exchange and Solvent Extraction"*, Vol. 6, p. 1, J. A. Marinsky and Y. Marcus, Eds., Marcel Dekker, New York
SMITH, R. L. (1971), "Excretion of drugs in bile", in: *Concepts in Biochemical Pharmacology*, Part 1, p. 354, B. B. Brodie and J. R. Gilette, Eds., Springer Verlag, Berlin, New York
SUGIYAME, T., Y. SUZUKI and T. TAKEUCHI (1973a), *J. Chromatog. 77*, 309
SUGIYAME, T., Y. SUZUKI and T. TAKEUCHI (1973b), *J. Chromatog. 80*, 61
SUGIYAME, T., Y. SUZUKI and T. TAKEUCHI (1973c), *J. Chromatog. 85*, 45
SWAN, D. F. K. (1972), *Column 14*, 9
VANDERHEEREN, F. A. J., D. J. C. J. THEUNIS and M. T. ROSSEEL (1976), *J. Chromatog. 120*, 123
VIDIC, H. J., DROSS and H. KEWITZ (1972), *Z. Klin. Chem. Klin. Biochem. 10*, 156
WELCH, R. M., R. L. DEANGELIS, M. WINGFIELD and T. W. FARMER (1975), *Clin. Pharmacol. Ther. 18*, 249

3

Determination of thiazinamium sulphoxide cations in body fluids

Contents

Summary

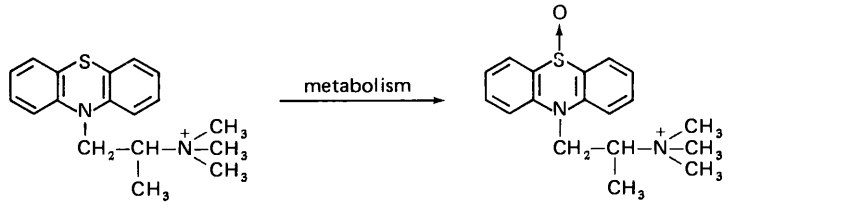
A sensitive and selective method is described for the isolation from urine and bile and quantitation of the extremely water soluble cation thiazinamium sulphoxide (metabolite of thiazinamium cation, mostly administered as methylsulphate). The isolation method is based on column chromatography with Amberlite XAD-2® followed by two-dimensional thin-layer chromatography. The spots are visualized by immersion in an oxidative mixture and quantitation is done by measuring the transmission of the spots with the aid of a "flying spot" densitometer.

Application of the method for urine and bile is described, the detection limit being 40 ng/ml.

A recovery of 90.6 ± 6.5 (S.D.)% for urine and 96.1 ± 4.8 (S.D.)% or 103.6 ± 7.8 (S.D.)% for bile, depending on the method used, was found.

3.1. Introduction

Thiazinamium sulphoxide cation is the only metabolite of thiazinamium cation (mostly administered as methylsulphate) found in man (Fig. 1).



THIAZINAMIUM CATION — I

THIAZINAMIUM SULPHOXIDE CATION — II

FIG. 1: *Formation of thiazinamium sulphoxide cation by metabolic oxidation.*

Being a quaternary ammonium compound, normal solvent extraction is impossible because the molecule is ionized at all pH values.
As described in Part II, Chapter 2, the parent compound can easily be isolated by ion pair liquid-liquid batch extraction, but attempts at isolating the sulphoxide cation by this technique failed. As mentioned earlier (Part II, Chapter 1) an ion pair extraction method can be used, provided the molecule consists of a sufficiently lipophilic moiety. This means that it should not contain any other strongly polar group besides the ionized group, which will be neutralized by the counter ion during the ion pair extraction. Thiazinamium cation contains a lipophilic phenothiazine nucleus, but apparently the lipophilicity of this moiety is strongly decreased by the oxidation of the sulphur atom yielding the sulphoxide.
We investigated, then, the applicability of Amberlite XAD-2® column chromatography. This macroreticular copolymer of styrene and divinylbenzene has successfully been used in the isolation of a wide variety of drugs and drug metabolites from urine and blood (BASTOS and others, 1973; WEISSMAN and others, 1974; MACHATA and VYCUDILIK, 1975). A comprehensive review of the applicability of Amberlite XAD-2® was given by SAMUELS BRUSSE and others (1974). As far as we know, however, its application to the isolation of quaternary ammonium compounds has so far never been described.
It was found by us that both thiazinamium and thiazinamium sulphoxide cations were adsorbed on the column when urine or bile samples were passed through an XAD-2 column (JONKMAN and others, 1976). Elution could be done by methanol (100%). Direct spectrophotometric quantitation in the methanol extract could not be carried out because of co-extraction of various other compounds from urine or bile.
For this reason a two-dimensional thin-layer chromatographic separation

was developed to separate the sulphoxide from its parent compound and from these pigments. After an oxidative colour reaction the spots could be quantitated by *"in situ"* transmission measurements.
Although the sensitivity of the method appeared to be sufficient for measuring concentrations of thiazinamium sulphoxide cations in urine and bile after a therapeutic gift of thiazinamium methylsulphate, the concentrations in plasma were too low to be measured with adequate precision.

3.2. Materials

We used the following materials for determination in both urine and bile.

Apparatus
All densitometric measurements were performed on a Vitatron TLD 100 "flying spot" densitometer using a filter of 525 nm (± 2 nm), and connected with a combined recorder-integrator Vitatron UR 402 (Vitatron, Dieren, The Netherlands).

Chemicals
Amberlite XAD-2®, particle size 300–1000 µm (Serva, Heidelberg, G.F.R.), purified by extraction in a Soxhlet apparatus with methanol during 3 hours and stored in a refrigerator (4° C) under a methanol/water mixture (30 : 70).
Methanol, ammonium acetate, ammonia 25%, ferric chloride, acetone, ethanol 100% and sulphuric acid were reagent grade (Pro Analysi®, E. Merck, Darmstadt, G.F.R.) and were used without purification.
Solutions of thiazinamium sulphoxide iodide containing 0.5, 1.0 or 2.0 µg/20 µl of methanol were used as references. The pure substance for these reference solutions was obtained from SPECIA, Rhône Poulenc, Paris, France, or prepared by ourselves in the following way.
Principle: promethazine is oxidized by means of hydrogen peroxide to promethazine sulphoxide. This is converted into thiazinamium sulphoxide by means of methyl iodide.
Procedure: 1 g of promethazine.HCl was dissolved in 2.5 g of acetic acid and 1.5 ml of water. Then 0.36 g of hydrogen peroxide (30%) was added dropwise. The mixture was heated to 40° C and again 0.36 g of hydrogen peroxide (30%) was added dropwise. The temperature was then raised to 60° C and was maintained at this value for 1 hour; next 2.0 g of sodium hydroxyde in 5.0 ml of water was added and the aqueous phase was extracted four times with a portion of 3.5 ml of dichloromethane.

The dichloromethane was evaporated under reduced pressure in a Rotavapor® apparatus at room temperature and the residue was recrystallized from 6 ml carbon tetrachloride. Result: 94% yield of promethazine sulphoxide base.
500 mg of this substance was dissolved in 20 ml of acetone, after which 0.10 ml of methyl iodide, dissolved in 5 ml of acetone, was added and allowed to react during 72 hours at room temperature.
A white crystalline reaction product (thiazinamium sulphoxide iodide) precipitated and this was separated from the acetone by filtration. The product was dried in the air until constant weight was reached. Identification was performed by means of U.V. and I.R. spectra. By thin-layer chromatography the product was found to be chemically pure for at least 99%. Melting point: 253–256° C. Yield 71%.
Thin-layer plates: Fertig platten Kieselgel 60 from E. Merck, Darmstadt, G.F.R. (= silica thin-layers, 0.25 mm on glass plates 200 × 200 mm, without fluorescence indicator).

Glass ware
Glass columns of 15 cm length, internal diameter 8 mm and provided with a stopcock, were used.

3.3. Determination of thiazinamium sulphoxide cations in urine

3.3.1. Introduction

Although the concentration of thiazinamium sulphoxide cations in urine is dependent on several factors (dose, route of administration, liver function of the patient *etc.*) the method described below could be used successfully in all samples under examination.

3.3.2. Methods

Column chromatographic procedure

The glass column was provided with a glass wool layer of 0.5 cm at the bottom and filled with Amberlite XAD-2® suspension to a height of 6 cm. The column was washed with small portions of distilled water from a

separation funnel until approximately 30 ml of water had passed the column. Next 1.0 ml of urine (or a volume required to get a concentration of thiazinamium sulphoxide - calculated as iodide - of 0.5 to 2.0 µg in 20 µl of the final methanol solution) was transferred dropwise from a separation funnel on the column and allowed to pass through it with a velocity of appr. 10 drops per minute. The separation funnel was washed with 10 ml of distilled water and this was allowed to pass through the column. Finally the column was washed with appr. 50 ml of distilled water (20 drops per minute). The column was then allowed to dry and any remaining traces of water were removed by a stream of nitrogen. All aqueous eluates were discarded. The stopcock was closed and methanol transferred onto the column to a height of 2 cm above the resin. The adsorbent was vigorously stirred in the methanol by means of a metal rod in order to remove air bubbles and was then given 10 minutes to settle. Additional methanol was added from a separation funnel and at the same time eluted with a velocity of 1 ml per minute until a total of 15 ml of methanol had been collected in a graduated conical glass tube. Next, the methanol was evaporated in a water bath at 70° C, under a gentle stream of nitrogen. The residue was dissolved in methanol (200 µl).

Thin-layer chromatographic procedure

20 µl of the methanol solution was applied as a spot to a thin-layer plate with a 20 µl micropipette in the lower left hand corner at a distance of 2 cm from each side and the plate was developed in methanol to a height of 15 cm above the starting point in an unsaturated chamber. All developments of the plate and the drying procedures were carried out in the dark. After development the plate was dried at room temperature for 15 min. and the reference spots (0.5, 1.0 and 2.0 µg of thiazinamium sulphoxide iodide in 20 µl methanol) were applied to the plate above the spotting place of the sample. The plate was turned 90° and developed in a mixture of ammonium acetate (8 g), water (42 ml), methanol (200 ml) and ammonia (6 ml) to a height of 15 cm in an unsaturated chamber. The pH of this mixture is 9.0. The plate was dried during 15 min. at room temperature and immersed during 30 sec. in an oxidative mixture of water (150 ml), sulphuric acid (150 ml), ferric chloride (4 g), acetone (800 ml) and absolute ethanol (800 ml). This mixture was stable for 1 week. After immersion the rear side of the plate was rinsed with a sponge and dried with a towel. The plate was subsequently dried during 15 min. at room temperature and put in an oven (140° C) during 4 min. to develop the colours of the spots. After cooling the transmission of the pink spots was measured immediately on the thin-layer plate with the densitometer at a wavelength of 525 nm.

3.3.3. Results and discussion

Although thiazinamium cations are excreted in urine very fast, biotransformation occurs to a considerable degree (See Part III, Ch. 8-11). Evidently the sulphur atom in the phenothiazine group can be oxidized to the sulphoxide. The thiazinamium sulphoxide molecule contains two strongly polar groups, namely the quaternary ammonium group in the side chain and the sulphoxide group in the phenothiazine nucleus, thus causing an extremely good water solubility. Because of this apparent lack of a lipophilic moiety in the molecule an important prerequisite for ion pair extraction is possibly not fulfilled. The ion pair thiazinamium sulphoxide iodide has a water solubility of 24.5 mg/ml and is almost insoluble in 1,2-dichloroethane (see Table 1).

TABLE I: *Solubility of thiazinamium sulphoxide iodide in various solvents, determined according to the method described in Part I, 2.6. Concentrations were measured using the absorption maximum at 268 nm. With the chlorinated hydrocarbons this was not possible because the solvent itself absorbs light at this wavelength, in which cases the maximum at 295 nm was used.*

solvent	solubility (mg/ml)	solubility (mol/l)
water	24.5	5.54×10^{-2}
methanol	8.9	2.01×10^{-2}
ethanol 96% (v/v)	3.4	7.69×10^{-3}
ethanol 100%	1.0	2.26×10^{-3}
1-propanol	0.37	8.37×10^{-4}
chloroform	0.15	3.39×10^{-4}
dichloromethane	0.14	3.17×10^{-4}
1,2-dichloroethane	0.04	9.05×10^{-5}

However, we decided to investigate if any ion pair extraction is at all possible, using the same counter ions and extraction solvents as for thiazinamium cations (see Section 2.1.1.). The results of this preliminary

study indicate, that ion pair extraction was really impossible or only occurred to a very small extent.

Subsequently we tested freeze-drying of urine samples containing both thiazinamium and thiazinamium sulphoxide cations. The residue was dissolved in methanol, because this is a good solvent for thiazinamium sulphoxide cations (Table 1). The methanol solution was submitted to thin-layer chromatography in order to isolate thiazinamium sulphoxide cations from other components in the urine, including thiazinamium cations. Several thin-layer chromatographic procedures for the analysis of phenothiazine drugs as pure substances are described in the literature: NOIRFALISE (1964), FIKE (1966), KORCZAK-FABIERKIEWICZ and CIMBURA (1970), DE LEENHEER (1973) and FRENCH and others (1974). MACEK (1972) gave a comprehensive review of the literature on this subject. Only two of these authors also used thiazinamium. NOIRFALISE (*l.c.*) described thirty solvent mixtures of different composition but in none of these systems thiazinamium leaves the spotting place. DE LEENHEER (*l.c.*) gave a comprehensive description of thin-layer chromatography of forty-one drugs (mainly phenothiazine derivatives) and thirty-one metabolites (sulphoxides), including thiazinamium and thiazinamium sulphoxide. However, neither thiazinamium nor the sulphoxide did move in the two solvent systems described. COCHIN and DALY (1963) described the application of thin-layer chromatography for the analysis of phenothiazine derivatives and their metabolites in body fluids and tissues, but did not mention thiazinamium and thiazinamium sulphoxide.

We have tested several solvent systems and found (see below) that it was possible to separate the parent drug and the metabolite using this technique. However, this approach proved to be unsuccessful because of large amounts of interfering substances from urine.

We then decided to examine the cleaning of the methanol solution by means of column chromatography. A preliminary study was done with thiazinamium cation alone. Test solutions of thiazinamium methylsulphate in water or thiazinamium iodide in dichloromethane were transferred to a column. Several column materials were used, such as Al_2O_3 (acid, neutral or alkaline), silicagel and cellulose. Methanol, ethanol and 1-propanol were applied as mobile phases. However, in all cases the binding between the quaternary ammonium compound and the column material was almost completely irreversible.

We then investigated the applicability of Amberlite XAD-2® columns because this nonionic macroreticular adsorbent had proved to be useful in the bioanalysis. The molecules are bound to the hydrophobic surface of the resin by Van der Waals forces (GUSTAFSON and others, 1968). Amberlite XAD-2® is generally preferred to other apolar adsorbents like charcoal, because with the former the affinity of binding is rather low and reversible, which enables easy and efficient regeneration with organic solvents *e.g.*

methanol or a chloroform/iso-propanol mixture. We established that thiazinamium cation and thiazinamium sulphoxide cation are both very well adsorbed from urine and can be separated from many physiological components in this material in this way. Elution with methanol proved also to be almost complete at all pH values and as a result of this good reversibility Amberlite XAD-2® gave excellent recoveries, in contrast with the other column materials mentioned earlier.

It was found to be necessary for the Amberlite XAD-2® material to be purified before use by repeated extraction with methanol so as to remove disturbing strongly U.V. absorbing impurities from the synthetic resin, as was described by KELSEY and MOSCATELLI (1973). We performed this purification by continuous extraction during 3 hours in a Soxhlet apparatus provided with a metal sieve. The purified Amberlite XAD-2® was stored in a refrigerator (4° C) under a methanol/water (30 : 70) mixture, as was recommended by these authors.

When using this column chromatographic procedure, the parent drug and its metabolite were isolated from urine and were transferred into an organic solvent which enables a concentration by a factor up to 125. This concentrated extract, however, still contained a few interfering components from urine, so that direct U.V. measurement was impossible. Therefore a separation of the metabolite from the parent drug and from disturbing physiological components was necessary. Gas chromatography of thiazinamium is very well possible (especially as the iodide, see Chapter 2), but attempts at gas chromatographic quantitation of the sulphoxide were less successful. In our system (see Chapter 2) we found that when thiazinamium sulphoxide was injected into the gas chromatograph as iodide, demethylation occurred (just as happened with thiazinamium iodide) and promethazine sulphoxide was produced. KOFOED and others (1966) reported the separation between the pure substances promethazine and promethazine sulphoxide by means of gas chromatography.

These authors used short columns (60 cm), high oven temperature and a high air flow. The conditions necessary to obtain an acceptable retention time for the sulphoxide were such that the value for the parent compound became very low. This could cause difficulties when working with extracts of biological materials because these generally give rather large solvent peaks. We found that promethazine sulphoxide, formed in the injection port, appeared to possess rather low volatility and in addition on-column adsorption occurred. In the conditions which were necessary for a separation of promethazine (formed from thiazinamium) from the solvent peak, this resulted in rather long retention times for the sulphoxide (appr. 3 times that of the parent drug) and very broad peaks. This made accurate quantitation impossible.

Because in the above mentioned preliminary study we had found that separation between thiazinamium and thiazinamium sulphoxide cations

seemed feasible by means of a thin-layer chromatographic procedure, we investigated a combination of this technique and a preceding Amberlite XAD-2® column chromatographic process. The concentrated methanol extract obtained from this column chromatographic process was applied to a thin-layer plate and development was performed with mobile phases containing ammonium acetate, methanol and water. Optimum separation between thiazinamium and thiazinamium sulphoxide was obtained with a mixture of 8 g of ammonium acetate, 42 ml of water and 200 ml of methanol, to which 6 ml of ammonia was added (pH = 9). The R_f-values were 0.36 for thiazinamium sulphoxide and 0.54 for thiazinamium. But here as well there was too much interference of co-extracted compounds (channel formation). If this thin-layer chromatographic development was preceded by one with only methanol, however, the disturbing urine components moved with, or close to the solvent front, whereas thiazinamium and thiazinamium sulphoxide stayed at the starting point. This purification step and the actual separation step were combined into a two-dimensional procedure, because repeated development in one direction resulted in impaired migration of the spots.

After the first development each plate was spotted with three reference samples of thiazinamium sulphoxide iodide. In this way quality variation in the plates was easily corrected for and good accuracy and precision were warranted.

In thin-layer chromatography two possibilities exist for quantitative measurements. Firstly the spots can be scraped off and the drug can subsequently be removed from the silica by extraction, followed by measuring the concentration of the drug in the extract. The second possibility is an *"in situ"* measurement: a direct measurement of the spots on the plate. Because we had found – as mentioned above – an irreversible binding of the drug and its metabolite to silica gel, we opted for an *"in situ"* method. When applying an *"in situ"* method, two possibilities are available for quantitative determination of drug substances without fluorescence characteristics: reflection (or remission) and transmission. An advantage of reflection measurement is that the entire U.V. and visible light spectrum can be used. In general background noise is less in comparison to a transmission measurement. A disadvantage is the possible occurrence of light scattering. The detection limit depends upon the specific extinction of the drug substance, but is in general in the microgram range (JORK, 1968). Application of *"in situ"* reflection measurement of some phenothiazine derivatives (without thiazinamium methylsulphate) was described by EBEL and others (1974).

On the other hand, transmission measurement is restricted to light with a wavelength greater than 300 nm due to absorption characteristics of the glass plate and because silica gel G is more transparant at these wavelengths. Especially below 280 nm the attenuation of this adsorbent

rises rather steeply (GOODALL, 1973). An advantage of transmission measurement over reflection measurement for thiazinamium sulphoxide cation is a higher sensitivity of the former mentioned method (see below). A mathematical approach of densitometry was described by GOLDMAN and GOODALL (1968, 1970) and by SCHUTE and others (1970).
Because we observed that the sulphoxide seemed to accumulate at the surface of the glass plate rather than at the surface of the silica layer, we decided to apply the method of transmission measurement. The U.V. absorption spectrum (see Fig. 2) of thiazinamium sulphoxide iodide in water shows four maxima.

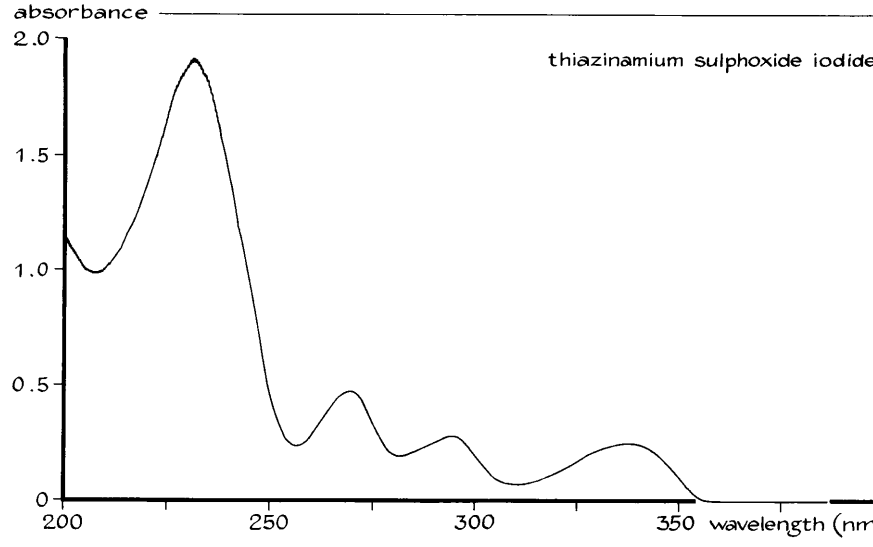


FIG. 2: *Ultra violet absorption spectrum of thiazinamium sulphoxide iodide in water (21.02 µg/ml; pH = 5.9; Unicam S.P. 800 Ultra Violet Spectrophotometer).*

Wavelength of maximum absorption, specific extinction and molecular extinction coefficients are given in Table 2. The highest absorption is obtained at a wavelength of 233 nm, but due to absorption by the glass plate this maximum cannot be used. As a matter of fact only the maximum at 338 nm can for this reason be taken into account, but at this wavelength the molecular extinction coefficient is so low that the sensitivity of the method falls below an acceptable level.
We then investigated the measurement of visible light absorption after colouring of the spots. When using a densitometric method for measuring of coloured spots the following prerequisites should be fulfilled (see also the review article by KIRCHNER, 1973 about quantitative thin-layer chromatographic analysis): a smooth application of the reagent is absolutely

TABLE 2: *Wavelengths (U.V. light) with maximum absorption* (λ), *specific extinction* ($E^{1\%}_{1\,cm}$) *and molecular extinction coefficients* (ε) *for thiazinamium sulphoxide iodide in water* ($pH = 5.9$).

λ (nm)	$E^{1\%}_{1\,cm}$	ε ($dm^3/mol \times cm$)
233	904	39952
268	228	10093
295	133	5888
338	114	5047

necessary; the colour obtained should be proportional to the amount of drug in the sample; the colouring reaction should be reproducible and the colour stable. In practice, it proved to be very difficult to apply a smooth layer of the reagent by spraying, and non-reproducible results were obtained with the reagents used (a mixture consisting of 2 ml of 37% formaldehyde (m/v), dissolved in 100 ml concentrated sulphuric acid and the reagent of CHAN and GERSHON; see below). Another possibility, of colouring the spots by means of vapour, was difficult for thiazinamium and its sulphoxide. With hydrochloric acid vapour both compounds became slightly red, but the spots were less intense than after spraying. Nitric acid vapour gave the same result but now the thiazinamium spot was not coloured at all. The accumulation of both substances at the surface of the glass plate rather than at the surface of the silica layer, can be an explanation for this obviously incomplete reaction.

We then examined application of the reagent by immersion of the plate in a tank for thin-layer chromatography. When using an immersion technique the water content of the immersion liquid should be as low as possible to avoid abrasion of the silica from the glass plate. We therefore decided to modify the spray reagent described by CHAN and GERSHON (1968), which they used for quantitative detection of chlorpromazine and its metabolites. The originally described reagent consisted of 0.5 g of ferric chloride, 18 ml of sulphuric acid and 150 ml of ethanol.

We found good results with an immersion reagent with rather high amounts of organic fluids (acetone and ethanol). The optimum composition was as follows: 4.0 g of ferric chloride, 150 ml of sulphuric acid, 800 ml of acetone, 800 ml of ethanol (100%) and 150 ml of water. After drying the plate was put in an oven for 4 min. at 140° C, which had proved a necessary step to increase the reaction rate, after which spots of pink colour appeared, with

an absorption maximum at 525 nm. These spots were measured by means of a "flying spot" densitometer. As described by several authors this principle offers advantages over measurements by means of a slit (SCHMIDTMAN and others, 1970; SCHUTE and others, 1970; DERTINGER and SCHOLZ, 1972; MLEKUSCH and others, 1973). The main advantage is a higher reproducibility because the result of the measurement is independent of non-homogeneous distribution of the substance in the spot.
The quantity in the sample can be calculated from the calibration curve, which was constructed from the reference on each plate. For practical reason we limited ourselves to three references. A larger number of spots could hardly be accomodated in consequence of channels formed by co-extracted compounds.
The parent drug thiazinamium cation can also be determined in most of the samples in this way but for routine analysis we preferred the quicker method as described in Part II, Chapter 2.

Selectivity, sensitivity, recovery and reproducibility of the procedure
When normal urine was examined no blank values were observed. So far no other drugs have been found to interfere with the present procedure. The volume of the sample taken under investigation depends on the concentration of the sulphoxide in urine after therapeutic use of the drug; it should be selected in such a way that the concentration in the final methanol solution is between 0.5 and 2.0 µg/20 µl (calculated as iodide). However, the volume should not be larger than approximately 25 ml, because in most instances of such large samples a large amount of compounds will be co-extracted, which results in a great residue and this will hamper complete re-uptake of the sulphoxide into the methanol. Moreover, it may cause difficulties during the spotting procedure.
The detection limit of thiazinamium sulphoxide (calculated as iodide) on the thin-layer plate is 100 ng, which results in a detection limit for the total procedure of 40 ng/ml urine. Accurate quantitation can be done at concentrations of 250 ng/ml and above.
The recovery was determined by using 1 ml of urine with a concentration of 10 µg/ml. Concentrations of about 10 µg/ml are often found in urine of patients after a therapeutic dose. The obtained value for the recovery was 90.6 ± 6.5 (S.D.) % (mean of seven determinations).
In the determination of concentrations of thiazinamium sulphoxide cations in urine a recovery of 90.6% was taken into account and all values obtained were corrected for it to 100% recovery.

Conclusion

The selectivity, sensitivity, recovery and reproducibility of the method described for the determination of thiazinamium sulphoxide cations in urine are such that they allow adequate studying of the urinary excretion of the metabolite after administration of a therapeutic dose of the drug.

3.4. Determination of thiazinamium sulphoxide cations in bile

3.4.1. Introduction

As regards the method described under 3.3.2., an essential difference between urine and bile is the high amount of physiological compounds of dark green colour in bile. However, this method is applicable also for bile after introducing a few modifications. For some samples a pre-extraction with light petroleum, boiling range 40–60° C proved to be necessary.

3.4.2. Methods

All bile samples were analyzed following the procedure described below under A. Unfortunately in a few instances of small volumes of concentrated bile interference occurred to an extent which hampers quantitative measurements of the spots. In these cases analysis was repeated employing the method described under B, which includes a pre-extraction.

A. A volume of bile up to a maximum of 0.5 ml was taken under investigation. The procedure as described under 3.3.2. was followed without alteration.

B. To 2.5 ml of bile 2.5 ml of water was added. Then 4.0 ml of light petroleum, boiling range 40–60° C, was added and the mixture was shaken three times for 20 sec. on a Vortex® mixer at maximum velocity. This was followed by centrifugation for 20 min. at 6000 *g*. The light petroleum layer and the intermediate layer were aspirated by means of a Pasteur pipette. Then, 1.5 ml of the remaining diluted bile was transferred to the column and the procedure was continued as described under 3.3.2.

3.4.3. Results and discussion

In general concentrations of thiazinamium sulphoxide cations in bile were found to be rather high (appr. 1 μg of thiazinamium sulphoxide - calculated as iodide - per ml bile and up). This allowed small samples to be taken and which could be diluted further with distilled water. As a result less interference of co-extracted compounds occurred in comparison to larger undiluted samples.
In cases of strongly concentrated bile samples, which often obviously contained a high amount of fat substances, only a column chromatographic procedure using Amberlite XAD-2® was not sufficient to purify the bile before it was applied to the thin-layer plate. The samples caused large green or green-brown background channels on the plate from the starting point to the front. Attempts to purify the bile by changing the pH of the washing solvent during the column chromatographic procedure were unsuccessful. Then we investigated the usefulness of a pre-extraction with a strongly lipophilic solvent, namely light petroleum, boiling range 40-60° C. As a result of this extraction part of the green coloured substances moved to the light petroleum. The fat substances were also largely extracted in this way. During centrifugation an intermediate layer of white or green colour was formed which was removed together with the light petroleum layer, and part of the remaining diluted bile was used for further examination. A single extraction with the solvent mentioned proved to give sufficient purification of the sample, without loss of recovery for the determination of thiazinamium sulphoxide cations.

Selectivity, sensitivity, recovery and reproducibility of the procedure
When bile samples were examined generally no disturbing spots were found on the place where the thiazinamium sulphoxide spot was expected.
The detection limit was found to be the same as described for the method for the determination in urine (3.3.3.).

TABLE 3: *Recovery of thiazinamium sulphoxide cations in the determination in bile. Concentrations calculated as the iodide. For methods used, see text.*

Method	initial concentration of thiazinamium sulphoxide		recovery mean ±S.D. %	number of determinations
	(μg/ml)	(mol/l)		
A	20.0	4.52×10^{-5}	103.6 ± 7.8	7
B	50.0	1.13×10^{-4}	96.1 ± 4.8	7

The recovery of the two procedures were tested using bile obtained from a patient before administration of the drug. Recovery in applying method A was determined by adding 1.0 ml of an aqueous solution of 25 mg of thiazinamium sulphoxide iodide dissolved in 100 ml of distilled water to a bile pool of 11.5 ml. The bile was subsequently divided into portions of 0.5 ml and in each portion the amount of thiazinamium sulphoxide cations was determined as described under 3.4.2. A. The results are given in Table 3. The recovery according to method B was determined as follows. The bile sample was divided into portions of 2.5 ml and to each portion 2.5 ml of an aqueous solution of thiazinamium sulphoxide iodide was added, to give a concentration of 50 μg/ml bile. The results are also given in Table 3. As can be seen from this table, for both methods recovery was found to be about 100%. In the determination of thiazinamium sulphoxide cations in bile a correction of the recovery to 100% was taken into account.

Conclusion

The two methods described for the determination of thiazinamium sulphoxide cations in bile give both good recoveries and are sufficiently reproducible. Pre-extraction with light petroleum did not significantly alter the recovery. The methods have sufficient sensitivity to study biliary elimination of the metabolite in an adequate way after administration of a therapeutic dose of thiazinamium methylsulphate.

References

BASTOS, M. L., D. JUKOFSKY and S. J. MULÉ (1973), *J. Chromatog. 81,* 93

CHAN, T. L. and S. GERSHON (1968), in: *"Quantitative Thin Layer Chromatography"*, p. 253, J. C. Touchstone, Ed., J. Wiley & Sons, New York

COCHIN, J. and J. W. DALY (1963), *J. Pharmacol. Exp. Ther. 139,* 160

DE LEENHEER, A. (1973), *J. Chromatog. 75,* 79

DERTINGER, G. and H. SCHOLZ (1972), *Pharm. Ind. 34,* 114

EBEL, S., B. DOBMEIER, M. FICK and H. KUSZMAUL (1974), *Arch. Pharmaz. 307,* 878

FIKE, W. W. (1966), *Anal. Chem. 38,* 1697

FRENCH, W. N., F. F. MATSUI, D. L. ROBERTSON and S. J. SMITH (1974), *J. Chromatog. 97,* 223

GOLDMAN, J. and R. R. GOODALL (1968), *J. Chromatog. 32,* 24

GOLDMAN, J. and R. R. GOODALL (1970), *J. Chromatog. 47,* 386

GOODALL, R. R. (1973), *J. Chromatog. 78,* 153

GUSTAFSON, R. L., R. L. ALBRIGHT, J. HEISLER, J. A. LIRIO and O. T. REID JR. (1968), *Ind. Eng. Chem., Prod. Res. Develop. 7,* 107

JONKMAN, J. H. G., J. WIJSBEEK, J. E. GREVING, R. E. VAN GORP and R. A. DE ZEEUW (1976), *J. Chromatog. 128,* 208

JORK, H. (1968), *J. Chromatog. 33,* 297
KELSEY, M. I. and E. MOSCATELLI (1973), *J. Chromatog. 85,* 65
KIRCHNER, J. G. (1973), *J. Chromatog. 82,* 101
KOFOED, J., C. FABIERKIEWICZ and G. H. W. LUCAS (1966), *J. Chromatog. 23,* 410
KORCZAK-FABIERKIEWICZ, C. and G. CIMBURA (1970), *J. Chromatog. 53,* 413
KORCZAK-FABIERKIEWICZ, C., J. KOFOED and G. H. W. LUCAS (1965), *J. Forensic. Sci. 10,* 308
MACEK, K. (1972), in: *"Pharmaceutical Applications of Thin-layer and Paper Chromatography"*, p. 171, Elsevier, Amsterdam
MACHATA, G. and W. VYCUDILIK (1975), *Arch. Toxicol. 33,* 115
MLEKUSCH, W., W. TRUPPE and B. PALETTA (1973), *J. Chromatog. 78,* 438
NOIRFALISE, A. (1965), *J. Chromatog. 19,* 68
SAMUELS BRUSSE, F., R. FURST and W. P. BENNEKOM (1974), *Pharm. Weekblad 109,* 921
SCHMIDTMAN, W., L. RESCHKE, L. BAUMEISTER and M. KOCH (1970), *Chromatographia 3,* 163
SCHUTE, J. B., H. J. DE JONG and H. A. DINGJAN (1970), *Pharm. Weekblad 105,* 1025
WEISSMAN, N., M. L. LOWE, J. M. BEATTE and J. A. DEMETRIOU (1974), *Clin. Chem. 17,* 875

Part III

Pharmacokinetics

1

Bioavailability - general remarks

Bioavailability is generally defined as *"the percentage of a drug contained in a dosage form that enters the systemic circulation in an unchanged form after administration of that dosage form"*.
Systemic or general circulation refers primarily to the venous blood (except the hepatic portal blood during the absorptive phase) and arterial blood which carries the drug to the tissues.
Other names commonly used for the same concept are: biological availability, physiological availability and systemic availability (WAGNER, 1971; BREIMER, 1976).
N.B. Some authors define bioavailability as "both the relative amount of drug from an administered dosage form which enters the systemic circulation and the *rate* at which this occurs". (*Guidelines for Biopharmaceutical Studies in Man*, 1972; GIBALDI and PERRIER, 1975; NOTARI, 1975; RITSCHEL, 1976).
In our studies the former definition will be used.
Complete entry in the general circulation is assured only when a dosage form is injected intravascularly. All other routes of administration (in our study intramuscular, oral and rectal) may result in appearance in the general circulation of only a fraction of the administered amount of the drug.
The concept of bioavailability of a drug does not take into account the fate of the drug after reaching the general circulation, such as distribution, biotransformation or excretion.
The bioavailability of a pharmacologically active ingredient of a medicament administered by a certain route is the result of several factors, for example the physicochemical properties of the drug molecule, formulation of the drug product, the condition of the patient, food intake and use of other drugs (WAGNER, 1971; BREIMER, 1973; RITSCHEL, 1973; SWARBRICK, 1973; CURRY, 1974; MERKUS, 1974; BREIMER, 1976; RITSCHEL, 1976).
Bioavailability can be determined in different ways. Measurements of concentrations of the drug in plasma from blood obtained from a systemic vein at different time intervals after administration of a single dose is the best method, provided that sufficient samples can be taken at the right time. Moreover the clearance pattern should be constant if different dosage forms for different routes of administration are tested.

Ideally the plasma concentration of the drug is followed until it has fallen to zero or up to the moment that reliable extrapolation to a plasma concentration of zero can be performed. In this way the total bioavailability can be measured. As another possibility one can measure the bioavailability during a certain time interval (as is shown for example in Chapter 4.). From the plasma concentrations obtained, a plasma concentration-time curve can be constructed on a linear scale. The area under this curve is an indication for the bioavailability of the drug. For quantitation of the bioavailability of a given dosage form it is necessary to compare the area under the plasma concentration-time curve after its administration with the values achieved with a reference standard. By definition intravenous injection of a drug guarantees a 100% bioavailability. If possible therefore, the area under the plasma concentration-time curve obtained after an injection in a peripheral vein should serve as a reference to determine the *absolute* bioavailability, as it is called, of a drug. In ideal circumstances the amount of drug administered by intravenous injection should be as large or approximately as large as the amount expected to come systemically available from the dosage form given by the route of administration for which the bioavailability is to be determined. If this leads to the necessity to use different doses for the two ways of administration, a correction in the calculation will be necessary to avoid mistakes. For quantitation of the absolute bioavailability the following equation can be used:

$$F_{abs} = \frac{(A.U.C.)_x}{(A.U.C.)_{i.v.}} \cdot \frac{D_{i.v.}}{D_x} \cdot 100\,\% \qquad \text{(XI)}$$

where:

F_{abs} = the absolute bioavailability as fraction (percentage) of the dose

$(A.U.C.)_x$ = the area under the plasma concentration curve after giving a drug by route x

$(A.U.C.)_{i.v.}$ = the area under the plasma concentration curve after giving the drug intravenously

D_x = the dose of the drug given by route x

$D_{i.v.}$ = the dose of the drug given intravenously

In our investigations x was the intramuscular injection.
Several methods are available for calculation of the area under the plasma concentration curves (RITSCHEL, 1976). In cases of computer-fitted curves,

the A.U.C. can easily be calculated by integration of the equation. We preferred the simple method of cutting and weighing of a standard type of paper.
In our experiments we studied the absolute bioavailability of an intramuscular dose of 6.25 mg of thiazinamium as compared to an intravenous injection of the same dose in the same patient. The result was called the absolute bioavailability of the intramuscular injection during the time of the experiment (= seven hours).
It is also possible to determine the *relative* bioavailability of a dosage form after a certain route of administration. In this case a comparison is made with a dosage form and a route of administration resulting in a known, but not necessarily 100%, bioavailability. In our investigations the intramuscular administration was used as a reference for all other application forms (oral and rectal).
For quantitation of the relative bioavailability equation (XII) can be used:

$$F_{rel} = \frac{(A.U.C.)_x}{(A.U.C.)_{st}} \cdot \frac{D_{st}}{D_x} \cdot 100\,\% \qquad \text{(XII)}$$

where:

F_{rel} = the relative bioavailability as fraction (percentage) of the dose

$(A.U.C.)_x$ = the area under the plasma concentration curve after giving a drug by route x

$(A.U.C.)_{st}$ = the area under the plasma concentration curve of the reference standard of known bioavailability

D_x = the dose of the drug given by route x

D_{st} = the dose of the reference standard.

Another approach to bioavailability determination, suitable especially for drugs which are largely excreted unchanged in urine, is collecting urine and measuring the amounts of drug in it until excretion has been completed. This method is often used to determine the relative bioavailability of a drug from a dosage form. If the drug is metabolized to a large extent, the amount of the metabolite(s) should also be taken into account. It should be mentioned, however, that in the case of a "first pass effect" (*i.e.* biotransformation of the drug before entering the general circulation) a correction should be made.
In our studies we used this method to determine the intraindividual and

interindividual variation in the bioavailability of thiazinamium methylsulphate after oral administration of a single dose.
A third method for the determination of the bioavailability (absolute or relative) is measuring the magnitude of the pharmacological or therapeutic responses. Of course in these instances it is necessary that the intensity of the action can be easily and accurately determined in a quantitative way and that a certain correlation between plasma concentration and effect exists. (*N.B.* The values for plasma concentration and effect have to lay within the linear inclining part of a dose-response curve.)
In our studies we also used this monitoring of pharmacological responses, by measuring the changes in the heart rate after oral and rectal administration of thiazinamium methylsulphate. Moreover, from clinical side (see thesis L. E. VAN BORK) the monitoring of the therapeutic effect took place by measuring the changes in lungfunction.
Finally, it should be mentioned that it is also possible to determine the bioavailability of a drug by measuring concentrations of the drug in saliva, provided that a good correlation exists between plasma and saliva concentrations at all plasma levels and during the whole course of an experiment. This method was not used by us.
Apart from the methods described above for the determination of the bioavailability after a single dose of a dosage form, it is also possible to estimate this factor by measuring "steady state" plasma or saliva concentrations or by measuring the cumulative renal excretion after prolonged use of the drug.
In this thesis bioavailability has been studied only after the administration of single doses. Bioavailability studies after prolonged use of the drug have not yet been carried out.

References

BREIMER, D. D. (1973), *Pharm. Weekblad 108,* 309

BREIMER, D. D. (1976), *Pharm. Weekblad 111,* 1121

CURRY, S. H. (1974), in: *"Drug Disposition and Pharmacokinetics"*, Blackwell Scientific Publications, Oxford

GIBALDI, M. and D. PERRIER (1975), in: *"Pharmacokinetics"*, Marcel Dekker Inc., New York, U.S.A.

"Guidelines for Biopharmaceutical Studies in Man" (1972), American Pharmaceutical Association, Academy of Pharmaceutical Sciences, Washington D.C., U.S.A.

MERKUS, F. W. H. M. (1974), *Pharm. Weekblad 109,* 496

NOTARI, R. E. (1975), in: *"Biopharmaceutics and Pharmacokinetics"*, Marcel Dekker Inc., New York, U.S.A.

RITSCHEL, W. A. (1973), in: *"Angewandte Biopharmazie"*, Wissenschaftliche Verlagsgesellschaft, Stuttgart

RITSCHEL, W. A. (1976), in: *"Handbook of Basic Pharmacokinetics"*, Drug Intelligence Publication, Hamilton, Illinois 62341, U.S.A.

SWARBRICK, J. (1973), in: *"Current Concepts in the Pharmaceutical Sciences-Dosage Form Design and Bioavailability"*, Lea & Febiger, Philadelphia, U.S.A.

WAGNER, J. G. (1971), in: *"Biopharmaceutics and relevant pharmacokinetics"*, p. 12. Drug Intelligence Publications, Hamilton, Illinois 62341, U.S.A.

2

Pharmacokinetics after intravenous injection in man

Contents

2.1. Introduction

As pointed out in Chapter 1 of this part of the thesis an intravenous injection was given to seven patients in order to determine the absolute bioavailability of thiazinamium methylsulphate after intramuscular injection of an identical dose. In literature only a few publications are found dealing with the plasma concentration-time curves in *man* after an intravenous injection of quaternary ammonium compounds.

SIDELL and GROFF (1971) have studied the pharmacokinetics after intravenous (and intramuscular) injection of the cholinesterase reactivator 2-pyridinium aldoxime methochloride (pralidoxime) in man. RAABLAUB and FREY (1972) described the pharmacokinetics of a bisquaternary ammonium compound, namely the muscle relaxant alcuronium dichloride (= dialkyl-nor-toxiferine) using a tritium labelled substance. CALVEY and associates (1972–1976) reported comprehensively about the pharmacokinetics after an intravenous bolus of the anticholinesterase agent edrophonium chloride, both in man and rat. This group employed an enzymatic method for the determination of the plasma concentrations in man, while ^{14}C-labelled drug substance was used in the animal experiments. SOMOGYI and others (1976) have studied in detail the pharmacokinetics of pancuronium bromide after intravenous injection in man.

BROEN CHRISTENSEN (1965) performed a study with decamethonium dibromide – also ^{14}C-labelled – in *animals* (rabbits). SUNDWALL and others

(1971) also studied the pharmacokinetics of a quaternary ammonium compound in animals after intravenous injection. This study concerned the anticholinergic quaternary ammonium compound emepronium bromide, which was investigated in dogs employing both a chemical (gas chromatography) and a radiochemical method (^{14}C-labelled drug substance). ALBANUS and others (1969) investigated plasma concentrations of ^{14}C-methylatropine in mice; unfortunately the sample schedule used does not allow adequate pharmacokinetic evaluation of their results (only information about the elimination-phase being presented).
All authors mentioned above reported a steep drop of the amount of drug in the plasma after the injection, resulting after a few hours in such low concentrations of the drug in plasma that they were hardly detectable.

2.2. Materials and methods

Subjects and conditions

Seven male patients suffering from generalized obstructive lung diseases (see thesis of L. E. VAN BORK) participated in this study. All patients had been given the entire information regarding the nature of the experiment and all had agreed to cooperate. The patients were aged between 28 and 63 (mean 48 ± 12 (S.D.) years) and their weights ranged from 67–85 kg (mean 78 ± 7 (S.D.) kg).
No abnormalities in their circulation, blood composition, kidney function, liver function or digestive tract were found.
The use of other drugs - which could have influenced the clinical or pharmacokinetic part of our investigations - was restricted as much as the condition of the patients permitted (*N.B.* Disturbance during the gas chromatographic procedure is an additional reason to avoid the use of promethazine hydrochloride; see Part II, Chapter 2.). In consequence none of the patients of this group used other drugs during the experiment and the week preceding it, except one (U.D.), who one time used a spray of salbutamol (Ventolin®) six hours before the experiment started.
Patients fasted overnight. Before giving the dose, a control blood sample was drawn and the urine bladder was emptied as completely as possible. The experiments commenced at appr. 9 a.m. The subjects were seated in a chair during the entire experiment, except during times of urine collection. The drug was administered by an injection stretched out over a period of

appr. 0.25 min. in a cubital vein in the left arm. Blood samples were drawn at 3, 6, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 330 and 420 min. after injection. The samples were appr. 10 ml each and were obtained from a permanent cannula (indwelling catheter Braunule T with LOK®, B. BRAUN, Melsungen, G.F.R.) with a K-75[a] three-way stopcock Pharmaseal® (Herstal, Belgium) placed in a cubital vein of the other arm. The blood was collected in glass tubes with screw caps (Sovirel®), each of which contained one drop of heparin solution (5 mg = 500 U of heparin sodium per ml of distilled water). The blood was immediately stored in a refrigerator (4° C) and centrifugated within two hours after collection for 20 min. at 6000 *g*. Next 4.0 ml of the plasma layer was transferred to a centrifuge tube of 50 ml capacity with Quickfit® stopper and stored at −20° C until the analysis was performed which always happened within two months.
During the entire experiment the heart rate was registered just before a blood sample was drawn. During the first 30 min. an electro-cardiogram was recorded (Officine Gallway, E.C.G. Model Cld). After that the heart rate was counted for 15 sec. and the heart rate in beats/min. was calculated. Heart rate was also recorded using a pulse monitor (Medical and Industrial Equipment Ltd., London, England).
Directly after injection each patient was allowed to drink 200 ml of water. After 90 and 210 min. the patients were given a light meal (rusks with sugar and a glass of orange lemonade). While the experiment was going on the patients were allowed to drink some water on request.
Urine was collected every hour if possible.

The dosage form

Amounts of basic drugs in dosage forms are frequently expressed as the amount of the base instead of the salt. *For the drug under study the dose was calculated as thiazinamium base (= hydroxyde) but it was administered as the methylsulphate.* This implies a factor of 1.297, due to difference in molecular weight.
As an intravenous injection 8.11 mg of thiazinamium methylsulphate was applied as 0.25 ml of a solution containing 32.44 mg of thiazinamium methylsulphate in 1.0 ml of a 0.45% sodium chloride solution to which an antioxidant (sodium metabisulphite, 1.25 mg/ml) and di-sodiumedetate (0.05 mg/ml), were added. This amount of drug substance equals a dose of 6.25 mg of the base (thiazinamium hydroxyde). *This dose will be referred to as the 6.25 mg dose.* The solutions were prepared and sterilized in the pharmacy of the Groningen University Hospital, from the commercially available drug substance, which was obtained from SPECIA, Rhône Poulenc, Paris, France. Test samples for analysis of the batches were taken at random. The amount of drug in the dosage form was determined by

direct spectrophotometry (see Part I, Section 2.3 and 4.4) or by amphimetric titration (see Part I, Section 4.7.2.). Occasionally the gas chromatographic method described in Part II, Section 2.4. was used. All analyses were performed in duplicate. The amount of drug in the solutions was found to be within 3.0% of the stated amount.

Determination of thiazinamium cations in plasma

The amount of drug in the plasma was determined as described in Part II, Section 2.3. and expressed as nanograms of thiazinamium methylsulphate per ml plasma.

2.3. Results and discussion

Determination of pharmacokinetic parameters using the NAFFIT-1 programme

The plasma concentration-time curves obtained in the seven patients are represented in Fig. 1. On the right hand of this figure, in the same sequence, the curves are given on a semi-logarithmic scale.
It is clear that the data do not fit into an one-compartment model.
The semi-logarithmic plots relating thiazinamium methylsulphate concentration to time therefore were resolved into two exponential components (later calculations using the NONLIN programme confirmed this assumption to be correct; see below). To this aim a calculation programme, named NAFFIT-1, developed in our Department, was used (GREVING, *Personal Communication,* 1976). This programme computes the least squares fit and is able to deal with up to three-compartment models (*e.g.* a three-compartment open model or a two-compartment open model with an absorption phase). Calculations were performed on a Hewlett Packard 25 calculator. Correlation coefficients were calculated according to the method described by SEDMAN and WAGNER (1976).
In these calculations the general equation was

$$c_p = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \qquad \text{(XIII)}$$

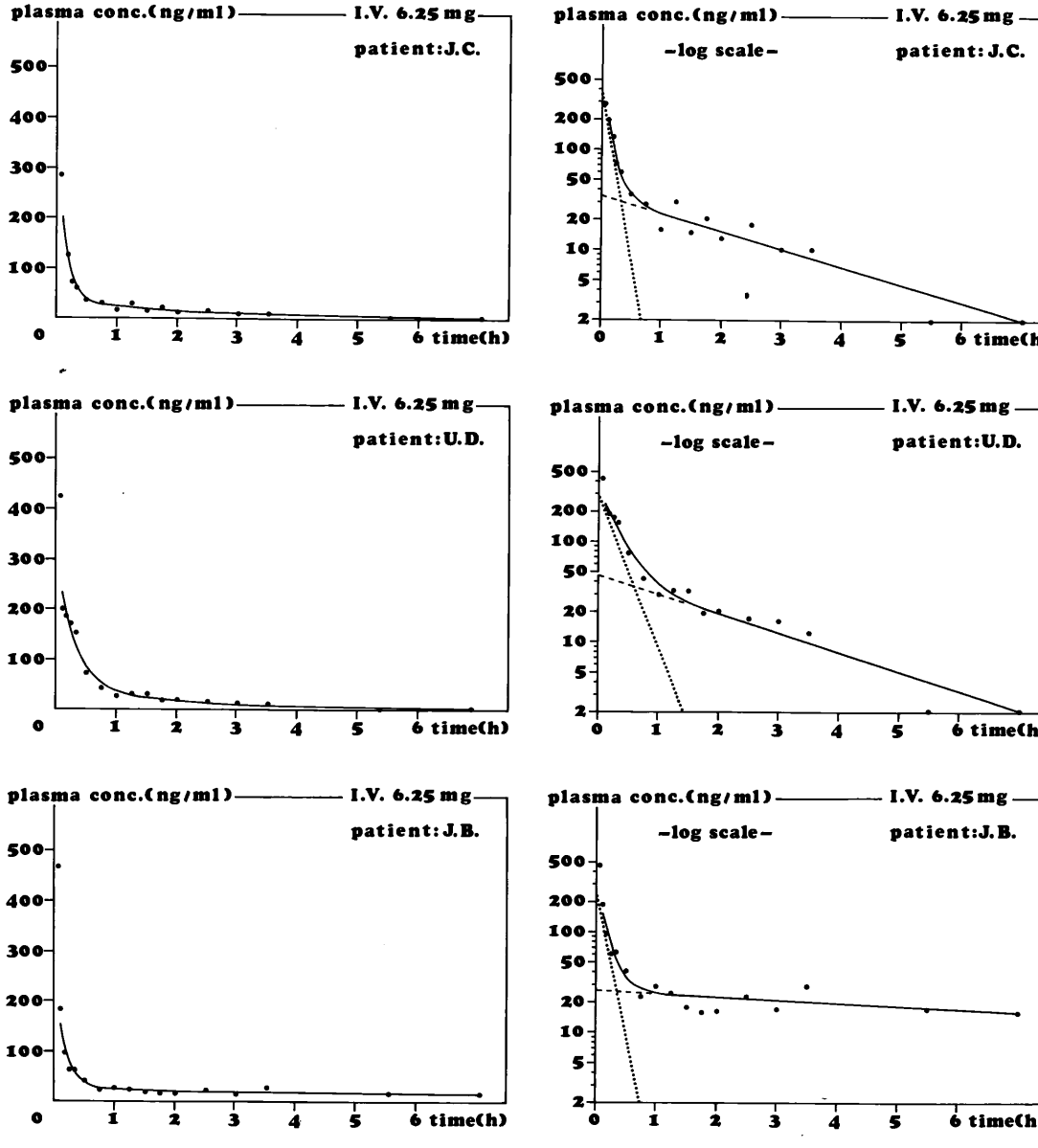
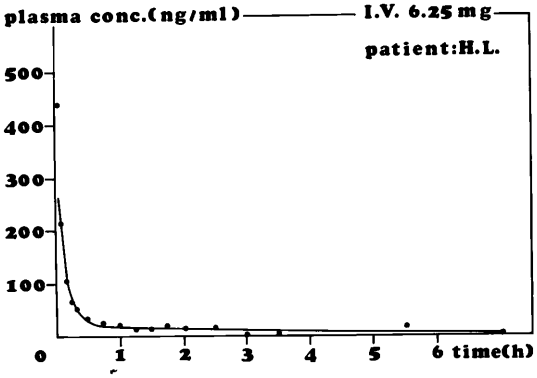
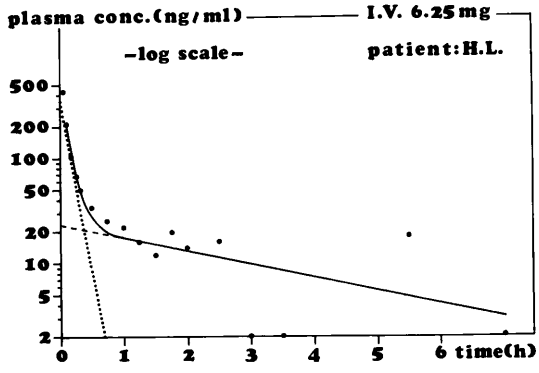
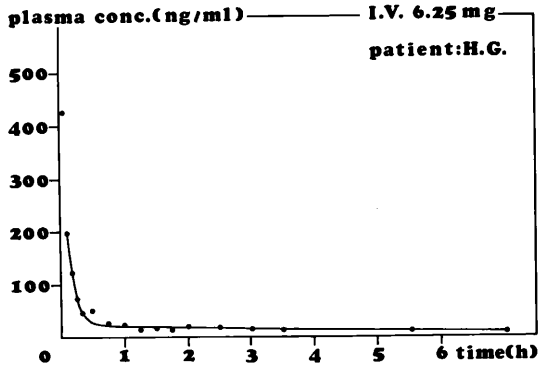
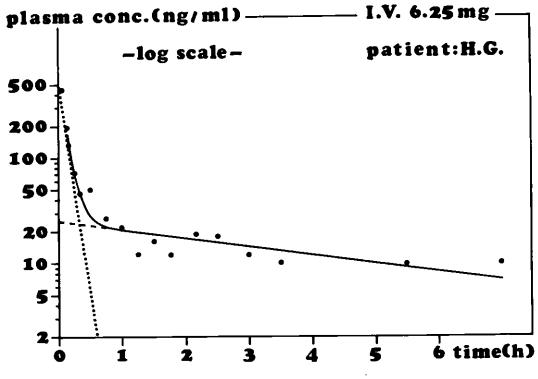


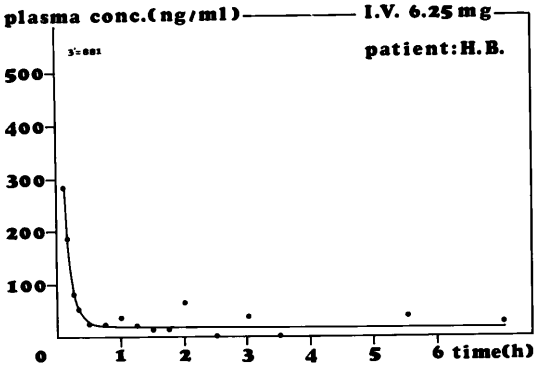
FIG. 1: *Plasma concentrations (ng/ml) after intravenous injection of a dose of 6.25 mg. NAFFIT-1 programme. On the left hand, the individual curves are given on linear scale; on the right hand the curves are given in the same sequence, but now on a semi-logarithmic scale.*

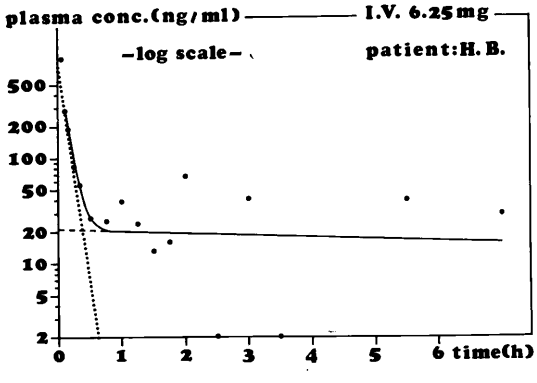

plasma conc.(ng/ml)
I.V. 6.25 mg
patient:H.L.
500
400
300
200
100
0
1
2
3
4
5
6 time(h)

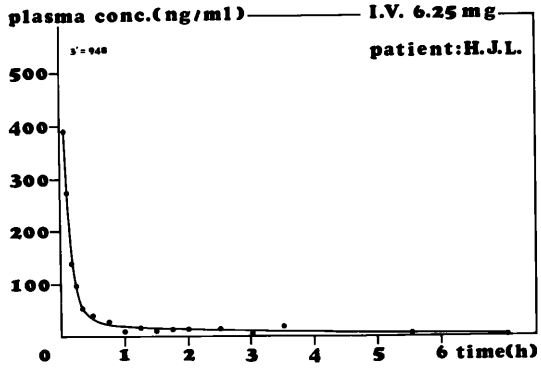

plasma conc.(ng/ml)
I.V. 6.25 mg
-log scale-
patient:H.L.
500
200
100
50
20
10
5
2
0
1
2
3
4
5
6 time(h)

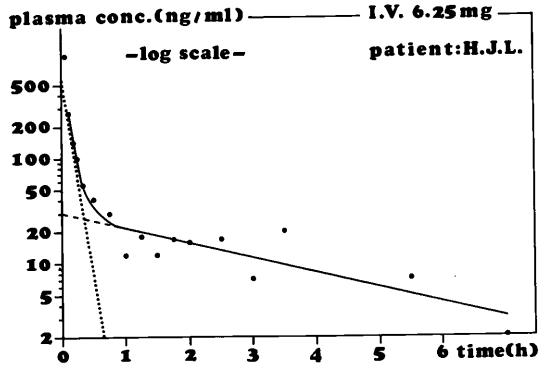

plasma conc.(ng/ml)
I.V. 6.25 mg
patient:H.G.
500
400
300
200
100
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.V. 6.25 mg
-log scale-
patient:H.G.
500
200
100
50
20
10
5
2
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.V. 6.25 mg
patient:H.B.
500
400
300
200
100
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.V. 6.25 mg
-log scale-
patient:H.B.
500
200
100
50
20
10
5
2
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.V. 6.25 mg
patient:H.J.L.
500
400
300
200
100
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.V. 6.25 mg
-log scale-
patient:H.J.L.
500
200
100
50
20
10
5
2
0
1
2
3
4
5
6 time(h)

where:

c_p = the concentration of thiazinamium methylsulphate in plasma at t min. after injection (ng/ml)
t = time after injection (min.)
A, α, B and β are described in Appendix 1.

After the intravenous injection in all seven patients thiazinamium cations were rapidly cleared from the plasma. The mean concentration of the drug in plasma decreased from 554 ± 254 (S.D.) ng/ml to 137 ± 36 (S.D.) ng/ml between 3 and 10 min. after injection. As these figures indicate, large individual differences were found among the seven patients at 3 min. after injection. A plausible explanation of this variation may be that the drug had not yet been completely distributed through the blood. GOLDSTEIN and others (1974) describe that thorough mixing takes several minutes: Evans blue, a dye that is almost wholly restricted to circulating plasma was not evenly distributed till several minutes had passed. CALVEY and his associates (1972–1976) also assume that the quaternary ammonium compound edrophonium chloride is not yet thoroughly mixed during the first three minutes after intravenous injection in man. RAABLAUB and FREY (1972) stated that mixing of hydrophilic compounds is completed within less than 10 min. For these reasons we decided to discard the 3 minute values in our calculations.

The rapid initial clearance from the plasma resulted in a relatively constant plasma concentration of about 50 ng/ml at 25 min. after injection. The initial phase of rapid elimination from the plasma was followed by a much slower decline during the further course of the experiment: in most of the patients the plasma concentrations slowly sank to zero or approximately zero within 420 min. In the semi-logarithmic curves this two-exponential decline can be seen more easily, suggesting an open two-compartment model.

From the values obtained from the curves of the best fit several pharmacokinetic parameters were calculated. For these calculations we used the equations given by RITSCHEL (1976) for an open two-compartment model with rapid intravascular administration. In absence of information to the contrary, it was assumed that drug elimination takes place exclusively in the central compartment (see below). It was also assumed that most of the drug is eliminated in unchanged form (which was found to be the case with thiazinamium cations after intravenous injection, see Part III, Chapter 8.). These equations are given in Appendix 1. A representation of the model is given in Fig. 2. The pharmacokinetic parameters obtained in this way are given in Table 1.

The figures for $(t_{\frac{1}{2}})_\alpha$ (a mean value of 6.2 min. with a standard deviation of 2.8 min.) clearly demonstrate the rapid fall in plasma concentration during the first phase (α-phase, lasting appr. 45 min.).

TABLE I: *Pharmacokinetic parameters of thiazinamium methylsulphate after intravenous injection of a dose of 6.25 mg in seven patients. (NAFFIT-1 programme).*

Patient		J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	mean ± S.D.	averaged plasma conc. curve
Age	(year)	56	28	51	49	63	38	49	48 ± 12	
Body weight	(kg)	85	85	83	67	75	75	78	78 ± 7	
Height	(m)	1.78	1.80	1.75	1.76	1.78	1.65	1.78	1.76 ± 0.05	
Dose*	(mg)	8.11	8.11	8.11	8.11	8.11	8.11	8.11	8.11	
Dose*	(mg/kg)	0.0953	0.0953	0.0977	0.121	0.108	0.108	0.104	0.104 ± 0.009	
c_p^o	(ng/ml)	395	312	259	369	512	752	592	456 ± 173	359
A	(ng/ml)	360	268	233	347	487	731	562	427 ± 177	336
α	($min.^{-1}$)	0.12787	0.05603	0.10296	0.12621	0.15661	0.15637	0.14567	0.12453 ± 0.03573	0.10137
B	(ng/ml)	35	44	26	22	25	21	30	29 ± 8	23
β	($min.^{-1}$)	0.00710	0.00756	0.00121	0.00463	0.00295	0.00062	0.00542	0.00412 ± 0.00273	0.00218
$(t_{\frac{1}{2}})_\alpha$	(min.)	5.4	12.3	6.7	5.5	4.4	4.4	4.8	6.2 ± 2.8	6.8
$(t_{\frac{1}{2}})_\beta$	(min.)	97.6	91.7	572.7	149.7	234.9	1121.4	127.9	342.3 ± 382.4	318.2
k_{12}	($min.^{-1}$)	0.06617	0.01977	0.08184	0.06977	0.10491	0.13251	0.07554	0.07864 ± 0.03491	0.06914
k_{21}	($min.^{-1}$)	0.01780	0.01440	0.01142	0.01188	0.01045	0.00497	0.01253	0.01192 ± 0.00391	0.00853
k_{13}	($min.^{-1}$)	0.05100	0.02942	0.01090	0.04919	0.04420	0.01951	0.06303	0.03818 ± 0.01875	0.02587
V_c	(l)	20.5	26.0	31.3	22.0	15.8	10.8	13.7	20.0 ± 7.2	22.6
V_c	(l/kg)	0.24	0.31	0.38	0.33	0.21	0.14	0.18	0.26 ± 0.09	
$(V_d)_\beta$	(l)	147.5	101.2	282.2	233.5	237.3	339.4	159.3	214.3 ± 83.1	268.3
$(V_d)_\beta$	(l/kg)	1.74	1.19	3.40	3.49	3.16	4.52	2.04	2.79 ± 1.17	
Cl_{tot}	(ml/min.)	1045.5	765.1	341.2	1082.2	698.4	210.7	863.5	715.2 ± 332.4	584.7
r (correlation coefficient)		0.992	0.982	0.960	0.979	0.991	0.972	0.993		0.995

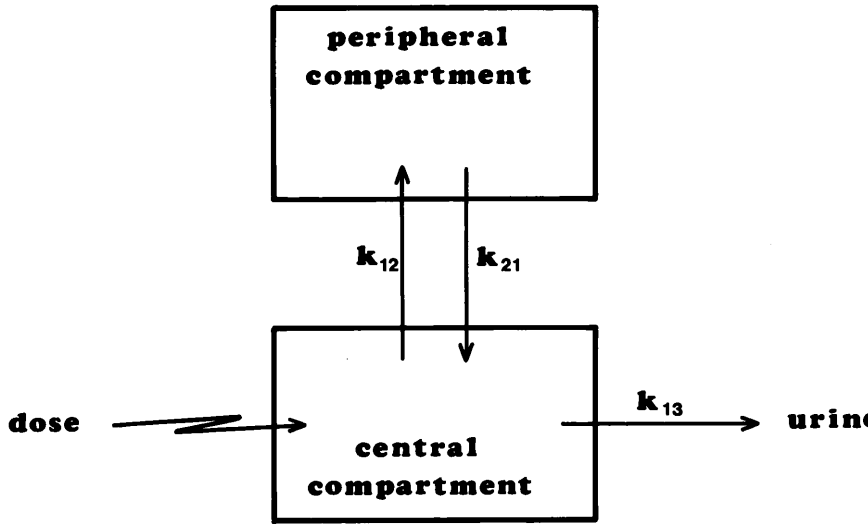


FIG. 2: *Schematic representation of an open two-compartment model with intravascular drug administration.*

The apparent volumes of distribution of the central compartment for the individual patients vary from 10.8 l to 31.3 l with a mean value of 20.0 ± 7.2 (S.D.) l. RITSCHEL (1976) stated that when a volume of distribution of a certain drug in an adult is found to be between 10–20 litres, or between 15–27 percent of body weight, it can be assumed that the drug is distributed only in the extracellular fluid. For most of our patients the value obtained is between these limits, suggesting that thiazinamium cations are mainly distributed in the extracellular fluid. This would be in agreement with the assumption that the strongly positive charge of the quaternary ammonium group would prohibit transport of thiazinamium cations through lipophilic cell membranes. In this respect it should be realized, that the values we obtained for the apparent volume of distribution are enlarged by the very rapid uptake of thiazinamium cations by the liver and kidneys, which was established in animal experiments (see Part III, Chapter 13).

Although it is dangerous to translate a pharmacokinetic parameter like the apparent volume of distribution into physiological terms, the arguments mentioned above suggest that thiazinamium cations are mainly distributed over extracellular fluids and maybe partly through the organs of elimination, which would mean that the central compartment would be comparable to extracellular fluids and liver and kidney.

Because distribution appeared to be the predominant process during the α-phase, this phase is also called the "distribution phase".

The distribution phase was followed by a second phase (β-phase) with a half-life $(t_{\frac{1}{2}})_\beta$ which is much longer than that found in the distribution phase. The half-life in the β-phase is generally considered to be the

biological half-life. The values we found ranged from 91.7 to 1121.4 min. with a mean value of 342.3 min. It should be realized, however, that this mean value has been greatly influenced by the extremely high values for patients H.B. and J.B. (1121.4 and 572.7 min. respectively) due to fluctuations in the final part of the curve. Discarding these values leads to a mean value of 140.4 ± 57.8 (S.D.) min. (an explanation for the fluctuations will be suggested below).

The apparent volumes of distribution of the β-phase (= the peripheral compartment) vary from 101.2 to 339.4 litres (mean 214.3 ± 83.1 (S.D.) l). Discarding the values of patients H.B. and J.B. the mean value was 176.0 ± 58.6 (S.D.) l. These values are much higher than the volume of the extracellular plus the intracellular fluids (appr. 40 l or 60% of the body weight). Because it is not likely, as stated above, that thiazinamium cations should penetrate to a large extent to the intracellular fluids, accumulation to a high degree in one or more organs or tissues resulting in local concentrations which are much higher than in plasma, can be an explanation for these rather large volumes of distribution. (*N.B.* As will be shown later the liver, kidney and mucus of the intestines may be responsible for this phenomenon).

The values found for the rate constants indicate that actually the drug is rapidly transferred from the central to the peripheral compartment, k_{12} being appr. 2 to 10 times greater than k_{21} (except again patient H.B., where this factor is 26). The rate constant for the elimination from the central compartment k_{13} is of the same order of magnitude as for the transfer between the central and the peripheral compartment.

Although the value for the half-life in the β-phase is rather long as compared to that of the α-phase, it should be realized that it is still very short in comparison to lipophilic drugs (PAGLIARO and BENET, 1975; RITSCHEL 1976, VAN ROSSUM, 1976). Short half-lives (appr. 1 to 2 hours) and rather small volumes of distribution (10 to 20 l) were also found for several penicillins, which likewise are rather hydrophilic drugs (NOTARI, 1975). As a consequence of the β-phase starting at rather low plasma concentrations (B between 21 and 44 ng/ml), only low plasma concentrations were found during this course of the experiment, and diminishing to almost zero at t = 420 min. In two cases (H.B. and to a lower degree in J.B.) distinct fluctuations were found in plasma concentrations. As a consequence the curve of the best fit is almost horizontal, which results in a very long half-life and a great volume of distribution as found by calculation from this curve. These fluctuations can be explained by assuming that part of the drug excreted in the bile is reabsorbed from the intestines (enterohepatic cycling). On the other hand accuracy of the method of determining thiazinamium cations in plasma is also lower in this concentration range. For these reasons the reliability of the pharmacokinetic parameters derived from the β-phase may be limited in some of the patients.

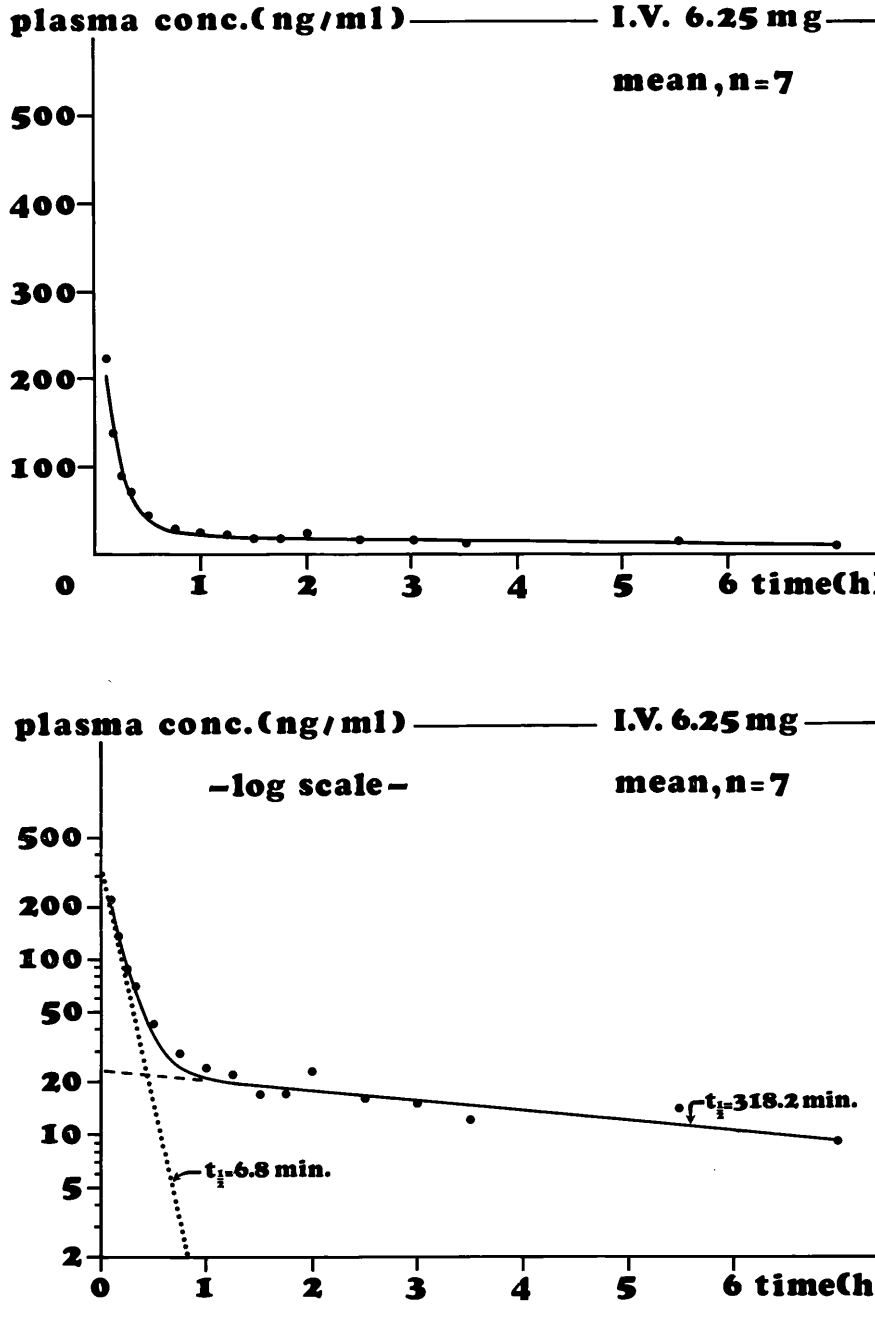


FIG. 3: *Plasma concentrations (ng/ml) after intravenous injection of a dose of 6.25 mg. NAFFIT-1 programme. The curve has been constructed from the averaged plasma concentrations of the seven patients. Above on linear scale, below on semi-logarithmic scale.*

Because elimination (excretion and metabolism) appeared to be the predominant process during the β-phase, this phase is also called the "elimination phase".
The total clearance for thiazinamium methylsulphate was found to be high. This term characterizes the clearing of a hypothetical plasma volume of a drug per minute. It comprises all pathways of excretion (renal, hepatic, metabolism). The mean value for the whole group is 715.2 ± 332.4 (S.D.) ml/min. Discarding the value for J.B. and H.B. a mean value of 893.7 ± 172.7 (S.D.) ml/min. was found, which are high values for plasma clearance.
As the normal glomerular filtration rate in the kidney is approximately 130 ml/min. (RITSCHEL, 1976), these values suggest that also other processes play an important role in the elimination of thiazinamium cations from plasma (*e.g.* active tubular secretion, biliary excretion, high extent of metabolism; this will be discussed in more detail in Chapter 6 to 12).
In this respect it is interesting to compare for some pharmacokinetic parameters the mean value, calculated from the values for the individual patients (Table 1) with the values for the same parameters but now calculated from the curve that was constructed from the averaged plasma concentrations (Fig. 3).
In the former case we found $(t_{\frac{1}{2}})_\alpha$ = 6.2 min., $(t_{\frac{1}{2}})_\beta$ = 342.3 min., V_c = 20.0 l and $(V_d)_\beta$ = 214.3 l.
In the latter case we found $(t_{\frac{1}{2}})_\alpha$ = 6.8 min., $(t_{\frac{1}{2}})_\beta$ = 318.2 min., V_c = 22.6 l and $(V_d)_\beta$ = 268.3 l. Except for the value for $(V_d)_\beta$, the difference is less than 10%.
Several authors (BREIMER, 1974; VAN ROSSUM, 1976) have stressed that erroneous values for pharmacokinetic parameters can be obtained from an average curve. They stated that calculation of the mean value of the individual parameters should be preferred, especially when there exists a large difference in the profiles of the individual curves (*e.g.* maximum plasma concentration appearing at different times after the administration). The good correlation we have found can be explained by the fact that the profiles of the individual curves are not essentially different in this group of patients.

The results of our study are in fair agreement with those reported for other quaternary ammonium compounds.
SIDELL and GROFF (1971) found for 2-pyridinium aldoxime methochloride (pralidoxime) a $(t_{\frac{1}{2}})_\alpha$ of appr. 8 min. and $(t_{\frac{1}{2}})_\beta$ of 74 min., which means that the half-life in the elimination phase is about 10 times the half-life in the distribution phase (for thiazinamium methylsulphate this ratio was found to be appr. 20).
RAABLAUB and FREY (1972) reported an α-phase for alcuronium (see Introduction) which lasts appr. 120 min. with a half-life of appr. 18 min.

(*N.B.* This figure was not mentioned by the authors, but calculated by us from their curves and values). The authors stated that the decline in plasma concentration in this phase is primarily caused by redistribution of the drug from the central to the peripheral compartment. This $(t_{\frac{1}{2}})_{\alpha}$ is substantial longer, but still in the same order of magnitude as found for thiazinamium methylsulphate. However, it should be noted, that in the alcuronium study the first blood sample was drawn 20 min. after injection, which seems to be a rather long interval for a hydrophilic drug. The α-phase was followed by a β-phase with a half-life of appr. 200 min. resulting in a ratio of 10 between $(t_{\frac{1}{2}})_{\alpha}$ and $(t_{\frac{1}{2}})_{\beta}$. Interesting are the findings of these authors in a patient with kidney malfunction (complete anuria). In this patient the half-life in the α-phase was the same as in a patient whose kidney function was normal, but the half-life in the β-phase was increased from appr. 3 to appr. 16 hours! The authors concluded that the kidneys fulfil a major clearing function during the β-phase (*N.B.* Alcuronium is excreted for 80 to 85% in urine and for 15 to 20% in bile with little or no entero-hepatic cycling or biotransformation).

CALVEY and associates (1976) also found a two-compartment model for edrophonium chloride; a rapid initial phase of distribution between 0 and 10 min. after injection with a $(t_{\frac{1}{2}})_{\alpha}$ = 0.54 to 1.92 min. was followed by a much slower decline ($(t_{\frac{1}{2}})_{\beta}$ = 24.23 to 45.00 min.) between 10 and 60 min. This means that for edrophonium chloride the half-life in both phases was even shorter than we found for thiazinamium methylsulphate.

After rigorous statistical analysis SOMOGYI and others (1976) found the plasma decay curve for pancuronium bromide to be biphasic, using the NONLIN least squares regression programme. As there was no obvious improvement in fit by the use of the three-compartment open model, the authors interpreted their data according to a two-compartment open model. The half-life of the α-phase was found to vary between 7.67 and 18.78 min. (mean value 12.46 ± 4.20 (S.D.) min.) and in the β-phase the half-life varied between 89.53 and 161.54 min. (mean value 132.53 ± 24.91 (S.D.) min.). Both values are comparable to those found for thiazinamium methylsulphate. However, we found the plasma clearance for thiazinamium methylsulphate to be substantially larger than that reported for pancuronium bromide. This can possibly be explained by the fact that for the latter compound renal clearance is the major pathway of elimination (AGOSTON and others, 1973; BUZELLO, 1975), whereas for thiazinamium methylsulphate besides a pronounced renal clearance, biliary clearance takes place to a considerable extent (see Part III, Chapter 9.).

TABLE 2: *Pharmacokinetic parameters of thiazinamium methylsulphate after intravenous injection of a dose of 6.25 mg in seven patients, NONLIN-programme. Weighting factor W = 1. For A, α, B and β the 95 % confidence limits are given according to univariate and support-plane.*

Patient	J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	mean ± S.D.	averaged plasma conc. curve
o (ng/ml)	349	271	384	480	381	692	584	449 ± 147	398
p									
A (ng/ml)	329	248	359	463	361	665	566	427 ± 146	375
univariate	260–398	205–292	238–481	343–584	290–432	423–907	444–692		334–415
s-plane	214–445	175–322	156–563	261–665	242–479	260–1071	360–776		307–442
: (min.$^{-1}$)	0.10393	0.04119	0.14464	0.15076	0.12221	0.15466	0.13738	0.12211 ± 0.03979	0.11095
univariate	0.08237–0.12550	0.02683–0.05556	0.10125–0.18803	0.11925–0.18227	0.10058–0.14384	0.10710–0.20221	0.11073–0.16404		0.09857–0.12334
s-plane	0.06782–0.14005	0.01713–0.06525	0.07198–0.21730	0.09799–0.20353	0.08599–0.15843	0.07502–0.23429	0.09274–0.18202		0.09021–0.13169
3 (ng/ml)	20	23	25	17	20	27	18	22 ± 4	23
univariate	14–25	11–36	20–31	11–22	15–25	16–39	11–24		19–26
s-plane	10–29	2–44	16–35	8–26	12–28	9–46	7–28		16–29
) (min.$^{-1}$)	0.00182	0.00385	0.00085	0.00106	0.00134	0.00022	0.00089	0.00143 ± 0.00117	0.00133
univariate	0.00095–0.00269	0.00070–0.00701	0.00029–0.00141	0.00049–0.00163	0.00078–0.00189	0.00004–0.00041	0.00039–0.00139		0.00099–0.00167
s-plane	0.00036–0.00327	−0.00142–0.00914	−0.00008–0.00180	0.00011–0.00201	0.00041–0.00227	−0.00009–0.00053	0.00005–0.00173		0.00076–0.00191
$t_{\frac{1}{2}})_\alpha$ (min.)	6.7	16.8	4.8	4.6	5.7	4.5	5.0	6.9 ± 4.4	6.3
$t_{\frac{1}{2}})_\beta$ (min.)	380.8	180.0	815.3	653.8	517.2	3150.0	778.7	925.1 ± 1006.2	520.5
ς_{12} (min.$^{-1}$)	0.07342	0.01543	0.12324	0.12034	0.09456	0.14319	0.10918	0.09705 ± 0.04229	0.08536
ς_{21} (min.$^{-1}$)	0.00767	0.00702	0.01021	0.00636	0.00768	0.00625	0.00510	0.00718 ± 0.00161	0.00766
ς_{13} (min.$^{-1}$)	0.02466	0.02259	0.01204	0.02512	0.02131	0.00545	0.02399	0.01931 ± 0.00757	0.01925
V_c (l)	23.6	30.3	21.4	17.1	21.6	11.9	14.1	20.0 ± 6.2	20.4
V_c (l/kg)	0.28	0.36	0.26	0.26	0.29	0.16	0.18	0.26 ± 0.07	
$V_d)_\beta$ (l)	319.1	178.0	303.2	405.8	343.1	294.1	379.4	317.5 ± 73.6	295.0
$V_d)_\beta$ (l/kg)	3.75	2.09	3.65	6.06	4.57	3.92	4.86	4.13 ± 1.23	
Cl_{tot} (ml/min.)	582.0	684.5	257.7	429.6	460.3	648.6	338.3	485.9 ± 159.6	392.3
r (correlation coefficient)	0.990	0.986	0.982	0.987	0.991	0.975	0.991		0.996

Determination of pharmacokinetic parameters using the NONLIN programme

The data of the experiments with intravenous administration have also been evaluated employing the NONLIN computer programme for nonlinear least squares determination of pharmacokinetic parameters (METZLER, 1969). The model and equations used were the same as described above. All data received a weighting factor of 1. The results are given in Table 2. (*N.B.* It should be observed that the NAFFIT-1 programme does not employ the concept of weighting factors).

Without having the intention to make a sophisticated statistical comparison of the two programmes used, a few conclusions become apparent. The correlation coefficients found in both programmes are almost identical. Also if we compare the values for A, α, B and β, which determine the shape of the plasma concentration-time curve, it is found that the calculated data using NAFFIT-1 deviate little from those calculated employing the NONLIN programme (see Table 3).

TABLE 3: *Comparison of some pharmacokinetic parameters as obtained with the NAFFIT-1 and NONLIN programme. In the latter a weighting factor of $W = 1$ is applied. Intravenous administration of 6.25 mg. The ratio NAFFIT-1/NONLIN is given, expressed as percentage.*

Patient	J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	averaged plasma conc. curve
c_p^o (ng/ml)	113	115	67	77	134	109	101	90
A (ng/ml)	109	108	65	75	135	110	99	90
α (min.$^{-1}$)	123	133	71	84	128	101	106	91
B (ng/ml)	175	191	104	129	125	78	167	100
β (min.$^{-1}$)	390	196	142	437	220	282	609	64

The deviation in the values for A and α are less than 35%. For most of the patients the values found for A and α by NAFFIT-1 are within the 95% confidence limits of the NONLIN calculation (both univariate and support-plane). The mean values of A and α were almost identical and proved to be not significantly different (Student's t-test: $p > 0.05$).

The difference between the values for B is larger, but for most patients the NAFFIT-1 estimates are again within the 95% confidence limits of NONLIN. On the other hand distinct differences are found for the β-values.

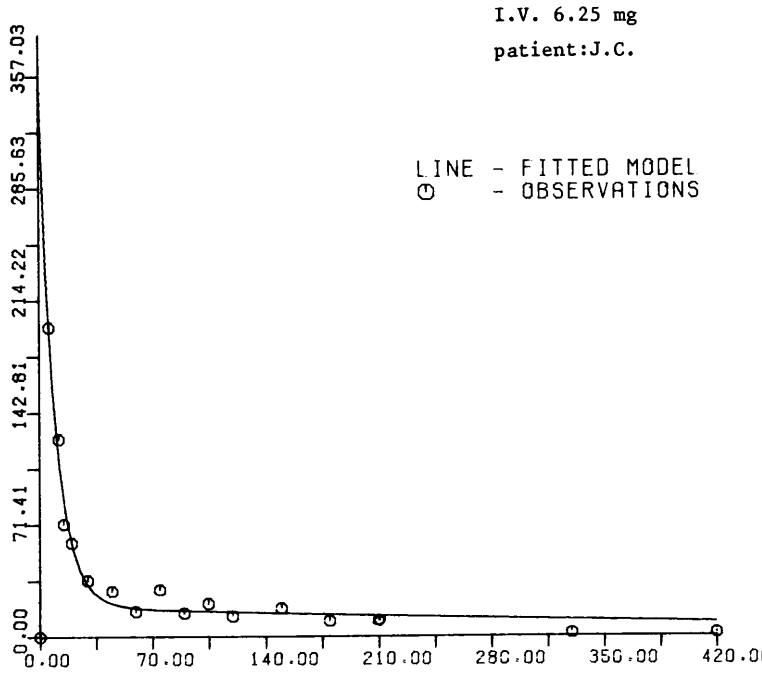


FIG. 4: *An example of a direct computer plot of a plasma concentration-time curve obtained after intravenous injection of a dose of 6.25 mg. NONLIN programme.*

However, the rather large interval between the 95% confidence limits indicates the reliability of the estimation of B and β with the NONLIN programme to be restricted, as already mentioned. The mean values for B proved to be not significantly different ($p > 0.05$), but mean values for β were found to be significantly different ($p < 0.05$).

Fig. 4 shows an example of a computer plot of a plasma concentration curve. In Fig. 5 and Fig. 6 the results of the calculations of NAFFIT-1 and NONLIN for the line of the best fit are given. Fig. 5 gives the curves for patient H.G. which patient was found to have the lowest degree of similarity between the two programmes. Fig. 6 represents the averaged plasma concentration curves for the seven patients. These curves have the best correlation between the two programmes. The figures show that, in general, the values for B and β are higher in the NAFFIT-1 calculation, which means that the $(t_{\frac{1}{2}})_\beta$ found, by NONLIN is larger (see Table 2).

When we compare the obtained figures for some other pharmacokinetic parameters, we find that, as expected, a good correlation between $(t_{\frac{1}{2}})_\alpha$ and V_c values and some difference between the $(t_{\frac{1}{2}})_\beta$ and $(V_d)_\beta$ values.

In conclusion it can be stated that, in general, the fit obtained by means of the NONLIN programme gives satisfactory results, assuming an open

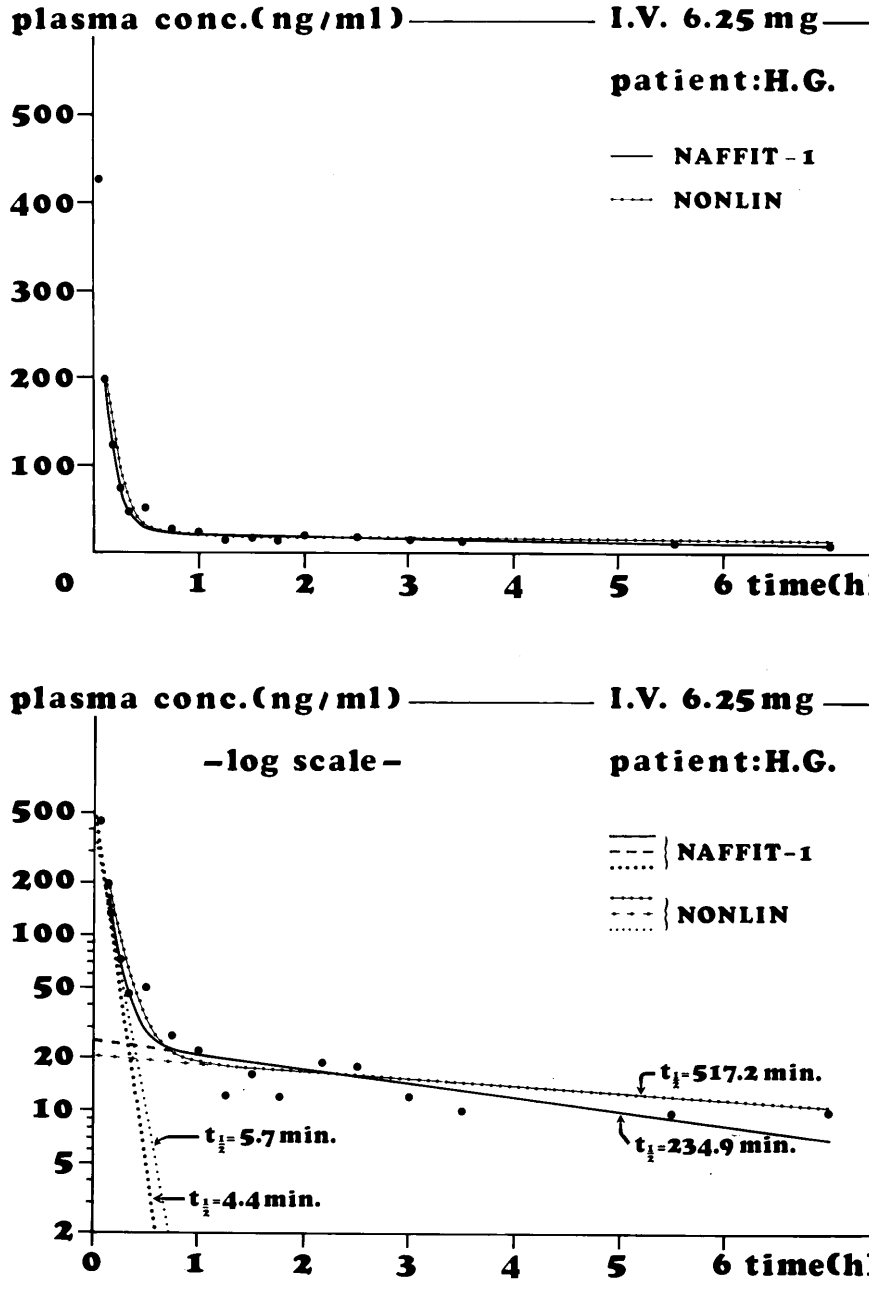


FIG. 5: *Plasma concentrations of patient H.G. after an intravenous injection of a dose of 6.25 mg. The curve of the best fit has been calculated, both by using the NAFFIT-1 and NONLIN programme. The above picture represents the curves on linear scale; below the curves on semi-logarithmic scale are given.*

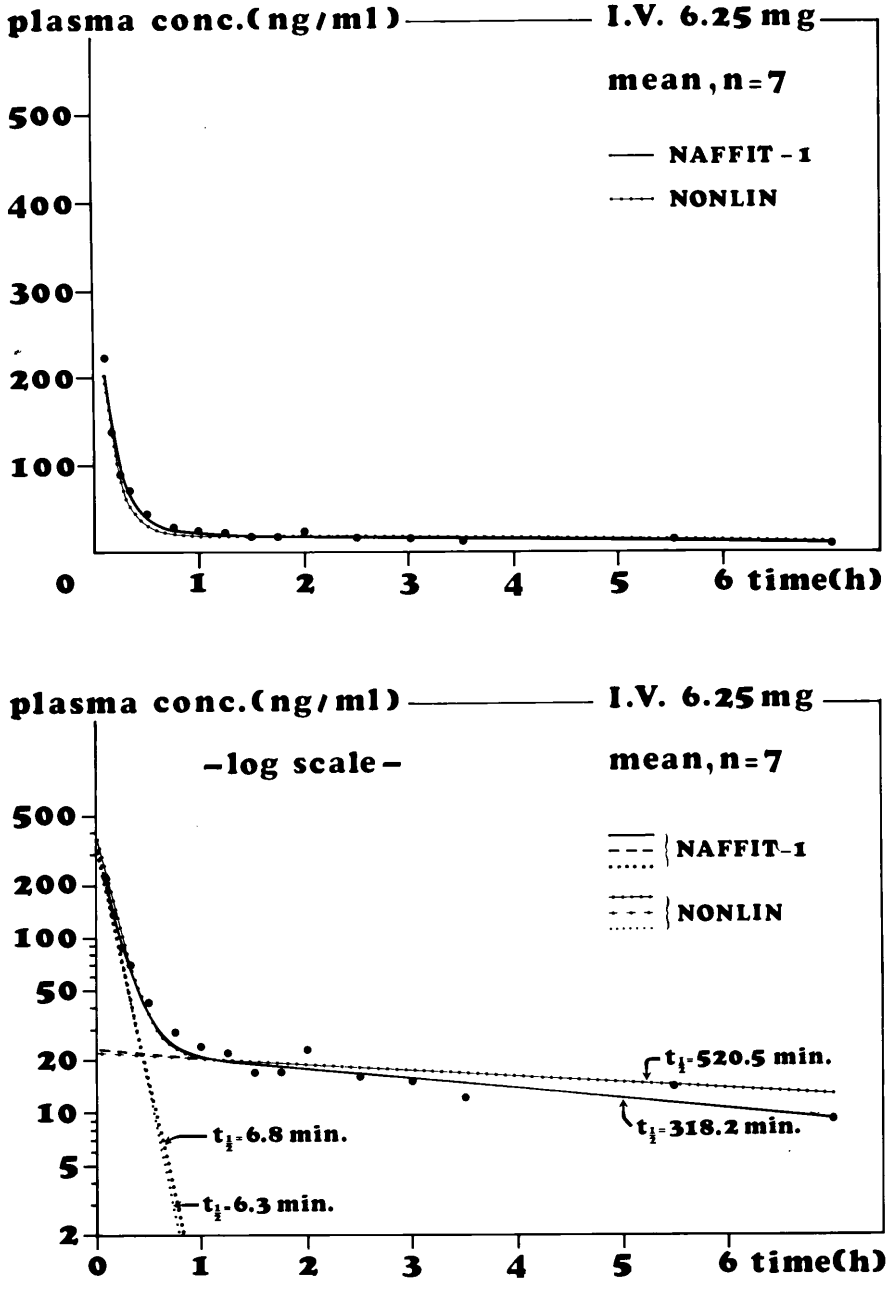


FIG. 6: *The curve of the averaged plasma concentrations of seven patients after given an intravenous injection of a dose of 6.25 mg. The curve of the best fit has been calculated both by using the NAFFIT-1 and NONLIN programme. The above picture represents the curves on linear scale; below the curves on semi-logarithmic scale are given.*

TABLE 4: *Pharmacokinetic parameters of thiazinamium methylsulphate after intravenous injection of a dose of 6.25 mg in seven patients. NONLIN programme. Weighting factor* $W = {}^1/\sqrt{y_i}$.

Patient		J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	mean ± S.D.	averaged plasma conc. curve
c_p^o	(ng/ml)	388	283	371	689	396	646	607	483 ± 160	404
A	(ng/ml)	354	249	344	640	371	630	579	452 ± 159	376
α	($min.^{-1}$)	0.12473	0.04806	0.14158	0.22659	0.13069	0.14023	0.14745	0.13705 ± 0.05207	0.11620
B	(ng/ml)	34	34	27	49	25	16	28	30 ± 10	28
β	($min.^{-1}$)	0.00643	0.00576	0.00144	0.01241	0.00339	0.00003	0.00511	0.00494 ± 0.00403	0.00306
$(t_{\frac{1}{2}})_\alpha$	(min.)	5.6	14.4	4.9	3.1	5.3	4.9	4.7	6.1 ± 3.7	6.0
$(t_{\frac{1}{2}})_\beta$	(min.)	107.8	120.3	481.3	55.8	204.4	23100.0	135.6	184.2 ± 153.3[1]	226.5
k_{12}	($min.^{-1}$)	0.06661	0.01745	0.11386	0.10963	0.08388	0.13556	0.07635	0.08619 ± 0.03865	0.07574
k_{21}	($min.^{-1}$)	0.01680	0.01084	0.01164	0.02764	0.01143	0.00350	0.01168	0.01336 ± 0.00740	0.01090
k_{13}	($min.^{-1}$)	0.04775	0.02553	0.01752	0.10173	0.03877	0.00120	0.06453	0.04243 ± 0.03330	0.03262
V_c	(l)	20.9	28.7	21.9	11.8	20.5	12.6	13.4	18.5 ± 6.2	20.1
V_c	(l/kg)	0.25	0.34	0.26	0.18	0.27	0.17	0.17	0.23 ± 0.06	
$(V_d)_\beta$	(l)	155.2	127.0	265.9	96.5	234.2	502.6	168.7	221.4 ± 137.2	214.0
$(V_d)_\beta$	(l/kg)	1.83	1.49	3.20	1.44	3.12	6.70	2.16	2.85 ± 1.84	
Cl_{tot}	(ml/min.)	998.0	732.7	383.7	1200.4	794.8	15.1	864.7	712.8 ± 396.7	655.7
r (correlation coefficient)		0.992	0.985	0.983	0.996	0.992	0.974	0.993		0.997

[1] = without patient H.B.

two-compartment model and all data receiving a weighting factor of W = 1. These results are in good agreement with those obtained by the NAFFIT-1 programme, except for the values of β.

In this respect, it is of interest to investigate if an improvement of the fit could be obtained by introducing different weighting factors. DANIEL and WOOD (1971) have indicated that the method of non-weighted least-squares analysis is statistically valid, only when all observations (y_i) have the same variance. If not, each point should be weighted inversely as its variance (BOXENBAUM and others, 1974).

Mathematical analysis of the conditions of the experiment (*e.g.* the method of determination of thiazinamium cations in plasma, blood sampling) pointed out that the variance in the observed values (y_i) is proportional to $\sqrt{y_i}$ (DE GRAAF, *Personal Communication*, 1976). Therefore, we have repeated the calculations using weighting factors of $W = 1/\sqrt{y_i}$. The results are shown in Table 4. In Table 5 these results are compared with those obtained using a weighting factor of W = 1. The correlation coefficient r and the sum of weighted square deviations (s.w.s.d.) have been used as criterion for exactness of the fit. (Manual of the NONLIN programme; BOXENBAUM and others, 1974; SOMOGYI and others, 1976). Table 5 shows that in all patients a slightly better fit was obtained when using weighting factors of $W = 1/\sqrt{y_i}$. As was expected the values for B and β are higher, because the low values of the plasma concentrations at the end of the curve now contribute to a higher extent to the calculated fit.

TABLE 5: *Comparison of the fits of a two-compartment model using weighting factors of W = 1 and $W = 1/\sqrt{y_i}$. The latter was also used for a three-compartment model. (r = correlation coefficient; s.w.s.d. = sum of weighted square deviations). NONLIN programme.*

Patient	J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	averaged plasma conc. curve
2-comp. model, W = 1								
r	0.990	0.986	0.982	0.987	0.991	0.975	0.991	0.996
s.w.s.d.	854	2172	1011	1096	725	4167	1311	401
2-comp. model, $W = 1/\sqrt{y_i}$								
r	0.992	0.985	0.983	0.996	0.992	0.974	0.993	0.997
s.w.s.d	294	821	604	258	396	3451	496	169
3-comp. model, $W = 1/\sqrt{y_i}$								
r	0.992	0.985	0.994	0.998	0.996	0.973	0.997	0.999
s.w.s.d.	313	822	262	218	216	3275	312	60

Using the latter method, the way of calculating the best fit with the NONLIN programme becomes more equal to the NAFFIT-1 programme and as a consequence the results achieved with both programmes show a higher degree of similarity now: the mean values for A, α, B and β are not significantly different (Student's t-test: $p > 0.05$).
Subsequently we have made a comparison between the results obtained by fitting a two- and a *three-compartment open model* to the experimental data, again using a weighting factor of $W = 1/\sqrt{y_i}$. Table 5 shows, that, for three patients (J.C., U.D. and H.B.) the fit according to a three-compartment open model is slightly inferior or equal to that obtained with a two-compartment open model. For the data of the other patients a small improvement was obtained in the fit assuming a three-compartment model. Fig. 7 shows the plots obtained with the two models for the averaged plasma concentrations. Apparently both models fit the data very well and no obvious improvement in fit by use of the three-compartment open model could be found.
As it is common practice in pharmacokinetic studies to utilize the simplest model of those that give satisfactory fits, we have therefore selected the two-compartment open model for further evaluation.

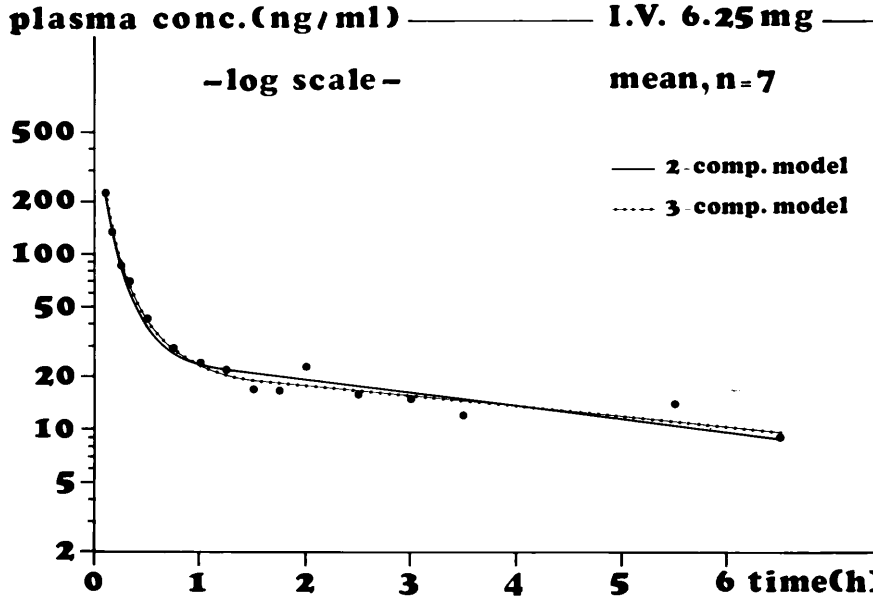


FIG. 7: *Semi-logarithmic plot of predicted two-compartment and three-compartment plasma concentration-time profiles and of experimental plasma concentrations (solid circles) for the averaged data. Intravenous injection of a dose of 6.25 mg. NONLIN programme.*

Conclusion

The plasma concentration-time curve of thiazinamium methylsulphate after an intravenous injection in man could be resolved into two exponential components, suggesting a two-compartment open model. Pharmacokinetic parameters were calculated using the NAFFIT-1 and the NONLIN programmes and a comparison between these two programmes was made (in both cases with a weighting factor $W = 1$). A good agreement between the two programmes was found. Applying a weighting factor of $W = 1/\sqrt{y_i}$ in the NONLIN programme gave a slight improvement of the fit. The fit could not be improved substantially assuming a three-compartment open model.

A short α-phase (= distribution phase) was found with a mean half-life of appr. 6 min. and a small volume of distribution (appr. 20 l), probably due to redistribution of the drug from the central to the peripheral compartment. This phase was followed by a β-phase (= elimination phase) with a much longer half-life (appr. 300 to 600 min.) and a larger volume of distribution (appr. 200 to 300 l) and resulting in plasma concentrations approaching zero at the end of the experiment (*i.e.* after seven hours).

References

AGOSTON, S., G. A. VERMEER, U. W. KERSTEN and D. K. F. MEYER (1973), *Acta Anaesth. Scand. 17,* 267

ALBANUS, L., A. SUNDWALL and B. VANGBO (1969), *Acta Pharmacol. Toxicol. 27,* 97

BACK, D. J. and T. N. CALVEY (1972), *Br. J. Pharmacol. 46,* 355

BACK, D. J. and T. N. CALVEY (1974), *Br. J. Pharmacol. 51,* 61

BARBER, H. E., T. N. CALVEY, K. T. MUIR and K. TAYLOR (1976), *Br. J. Pharmacol. 56,* 93

BOXENBAUM, H. G., S. RIEGELMAN and R. M. ELASHOFF (1974), *J. Pharmacokin. Biopharm. 2,* 123

BREIMER, D. D. (1974), in: *"Pharmacokinetics of Hypnotic Drugs"*, (thesis), Drukkerij Brakkenstein, Nijmegen, The Netherlands

BROEN CHRISTENSEN, CHR. (1965), *Acta Pharmacol. Toxicol. 23,* 275

BUZELLO, W., (1975), *Anaesthesist 24,* 13

CALVEY, T. N., N. E. WILLIAMS, K. T. MUIR and H. E. BARBER (1976), *Clin. Pharmacol. Ther. 19,* 813

DANIEL, C. and F. S. WOOD (1971), in: *"Fitting Equations to Data"*, Wiley-Interscience, New York

GOLDSTEIN, A., L. ARONOW and S. M. KALMAN (1974), in: *"Principles of Drug Action"*, J. Wiley & Sons, New York, U.S.A.

GRAAF, R. DE (1976), *Personal Communication.* (Mathematisch-Statistische Adviesafdeling, Katholieke Universiteit Nijmegen, The Netherlands)

GREVING, J. E. (1976), *Personal Communication*

METZLER, C. M. (1969), *NONLIN; Technical Report no 7292/69/7292/005,* Pharmaceutical Research and Development Division, The Upjohn Company, Kalamazoo, Michigan, U.S.A.

NOTARI, R. E. (1975), in: *"Biopharmaceutics and Pharmacokinetics"*, Marcel Dekker Inc., New York, U.S.A.

PAGLIARO, L. A. and L. Z. BENET (1975), *J. Pharmacokin. Biopharm. 3,* 333

RAABLAUB, J. and P. FREY (1972), *Arzneimittel-Forsch. 22,* 73

RITSCHEL, W. A. (1976), in: *"Handbook of Basic Pharmacokinetics"*, Drug Intelligence Publication, Hamilton, Illinois 62341, U.S.A.

ROSSUM, J. M. VAN (1976), in: *"Farmacokinetiek"*, Cursus Post Academisch Onderwijs; K.N.M.P., The Hague, The Netherlands

SEDMAN, A. J. and J. G. WAGNER (1976), *J. Pharm. Sci. 65,* 1006

SIDELL, F. R. and W. A. GROFF (1971), *J. Pharm. Sci. 60,* 1224

SOMOGYI, A. A., C. A. SHANKS and E. J. TRIGGS (1976), *Europ. J. Clin. Pharmacol. 10,* 367

SUNDWALL, A., K. UTHNE and J. VESSMAN (1971), *Acta Pharmacol. Toxicol. 29,* 385

3

Pharmacokinetics after intramuscular injection in man

Contents

3.1. Introduction

As pointed out in the "General Introduction", intramuscular injections of thiazinamium methylsulphate are in frequent use, for both diagnostic and therapeutic use (VAN BORK and others, 1977a, 1977b).
The aim of the following study was to investigate the bioavailability of the drug after the intramuscular route of administration and to see if a correlation exists between the plasma concentrations and the clinical effect obtained (see VAN BORK, *l.c.*). Moreover we selected this route for use as the reference to estimate the relative bioavailability of other routes of administration. The factors that can influence the shape of the plasma concentration-time curve were subject of investigation, too.
In the past, the intramuscular route of drug administration was regarded to be rather non-problematic from a biopharmaceutical point of view. It was generally assumed that absorption is complete and rather rapid, plasma peak concentrations being reached within approximately one to two hours. Absorption may be delayed by giving special long-acting preparations, consisting of a relatively insoluble or slowly dissolving salt (or complex) of the drug substance, or by giving a suspension in a lipophilic depot vehicle (or pellet). Examples of drugs given as intramuscular injection with delayed action are insulin, penicillin, corticotrophin, chlorpromazine and fluphenazine. The factors which influence the bioavailability of a drug after an intramuscular injection have been comprehensively discussed by BALLARD and NELSON (1965), BALLARD (1968) and more recently by SCHOU (1971) and by GREENBLATT and KOCH-WESER (1976). They need not be repeated here.
After an intramuscular injection a series of processes take place simultaneously; diffusion in the tissue depot ("stirring") and passage through the capillary membrane from tissue to plasma are the most important of these. SCHOU (1971) stated that "lipid soluble substances are absorbed more rapidly than insoluble ones because lipid molecules and ions diffuse through the cells of the capillary wall, whereas lipid insoluble compounds and ions diffuse solely through the water-filled pores, which seem to cover about 0.2% of the total capillary surface. As the unionized form of weak acids and bases is usually lipid soluble, drugs of this type will be most readily absorbed when the pH of the solution favors the unionized form of the drug". This shows the total area of capillary membrane to which the drug is exposed and the solubility of the compound in interstitial fluid to be important factors which influence the rate of absorption (see also EDITORIAL, *Lancet*, 1975).
Apart from lipophilicity the capillary blood flow has been found to be an important factor in relation to the rate of absorption from the muscle. EVANS and others (1973) measured resting human muscle blood-flow in

three pairs of muscles (*gluteus maximus, vastus lateralis* and *musculus deltoideus*) and found that the blood-flow was greatest in the deltoid, intermediate in the thigh and least in the buttock. They concluded that the differences were large enough to affect the rate of absorption and peak plasma concentrations following intramuscular administration of drugs. REEVES and others (1974) supposed that thigh injections were better than buttock injections for the antibiotic agent cephacetrile.

In addition some authors recently pointed out that intramuscular administration is not as uncomplicated as formerly assumed and that rapid and complete absorption is not always guaranteed. DUNDEE and others (1974) demonstrated on diazepam that the injection technique as such is important. Injection into fat tissue may result in very poor absorption.

Employment of special vehicles - often containing propylene glycol - to dissolve drugs which are insoluble in water can also lead to poor absorption, as was demonstrated by GREENBLATT and others (1974) for chlordiazepoxide, by HILLESTAD and others (1974) for diazepam and by KARLSSON and others (1974) and WILDER and others (1974) for phenytoin. Upon injection in the tissue the drugs precipitate in the interstitial fluid. The precipitate will redissolve only slowly, which results in low plasma concentrations (KOSTENBAUDER and others, 1975).

With regard to the *bioavailability of quaternary ammonium compounds after intramuscular administration in man* only little information based on blood level data is available. SUNDWALL (1960) comprehensively studied the intramuscular administration of pralidoxime and described the plasma concentration-time curves he obtained. SIDELL and GROFF (1971) performed a study with the same cation and calculated some pharmacokinetic parameters. SUNDWALL and others (1973) studied plasma concentration-time curves after intramuscular administration of emepronium bromide (Cetiprin®) in man and related these to certain pharmacological effects.

Other authors compared the efficacy of oral and intramuscular doses of quaternary ammonium compounds by measuring pharmacological effects. An example can be found in the paper by MÖLLER and ROSÉN (1968) who monitored heart rate, near-point of accommodation and salivary secretion after intramuscular injection of three quaternary ammonium compounds.

In our investigations of various aspects of the intramuscular administration of aqueous solutions of thiazinamium methylsulphate we employed three different doses.

With a low sub-therapeutic dose of 6.25 mg the pharmacokinetics of the drug after intramuscular injection were compared with the pharmacokinetics after intravenous injection of the same dose in the same patients.

A dose of 12.5 mg was used in two different investigations with different aims. Firstly, a comparative study was carried out with a dose of 25.0 mg. This study was primarily performed to investigate if the plasma concentrations obtained after injection of 12.5 mg were sufficient to achieve an

adequate clinical effect. Secondly, a dose of 12.5 mg was used to allow quantitation of the bioavailability after rectal administration.
An injection of 25.0 mg was given twice to the same subjects, once with the subject being allowed to walk during the experiment and once with the subjects having to stay put. In this way the influence of muscle contraction in the thigh (the place of injection) was studied. A dose of 25.0 mg was also used for quantitation of the bioavailability after oral administration.
We used the plasma concentrations obtained to compare a few pharmacokinetic parameters.

3.2. Materials and methods

3.2.1. Investigations with a dose of 6.25 mg

Subjects and conditions

These investigations were done with the same group of seven male patients as described for the intravenous study (2.2.). This time, however, none of the patients used other drugs during the investigation or in the week preceding it. The injection was given in the right thigh (*rectus femoris*, which is situated near by the *vastus lateralis* and *vastus medialis*) in a period of appr. 0.25 min.

The dosage form

The same solutions as mentioned under 2.2. "The dosage form" were used. The right dose was obtained by adapting the volume injected.

Determination of thiazinamium cations in plasma

Analysis was performed as described in Part II, Section 2.3. The amount of drug in plasma was expressed as nanograms of thiazinamium methylsulphate per ml plasma.

Calculation of the pharmacokinetic parameters

Estimation of the pharmacokinetic parameters was performed by employing the same methods as described under Part III, Section 2.3., namely the NAFFIT-1 and NONLIN programmes.
Most of the conclusions were drawn from the results of the former method. The results obtained with both programmes were compared.

Determination of the bioavailability

The areas under the plasma concentration-time curves were determined by cutting and weighing of a standard type of high quality paper. Bioavailability was calculated as described in Part III, Chapter 1.

3.2.2. Investigations with a dose of 12.5 mg

3.2.2.1. Comparison with a dose of 25.0 mg
Nine male patients with generalized obstructive lung diseases participated in this study. For reasons to be mentioned later (Chapter 9 and 10) two of them had to be considered separately.
The resulting group of seven patients were aged between 22 and 62, mean 40 ± 16 (S.D.) years, and weighed from 68 to 82 kg, mean 76 ± 6 (S.D.) kg. No electro-cardiograms were recorded.
All other conditions and methods were identical to those described under 2.2 and 3.2.1.

3.2.2.2. Determination of the bioavailability after rectal administration
Seven male patients with generalized obstructive lung diseases participated in this study. They were aged between 26 and 71, mean 57 ± 15 (S.D.) years and weighed from 67 to 86 kg, mean 75 ± 6 (S.D.) kg.
In this study the patients stayed in bed during the first hour after administration of the drug.
No electro-cardiograms were recorded.
All other conditions and methods were the same as described under 2.2 and 3.2.1.

3.2.3. Investigations with a dose of 25.0 mg

3.2.3.1. Comparison with a dose of 12.5 mg
This is the follow-up of the experiment described under 3.2.2.1. Patients and conditions were exactly the same.

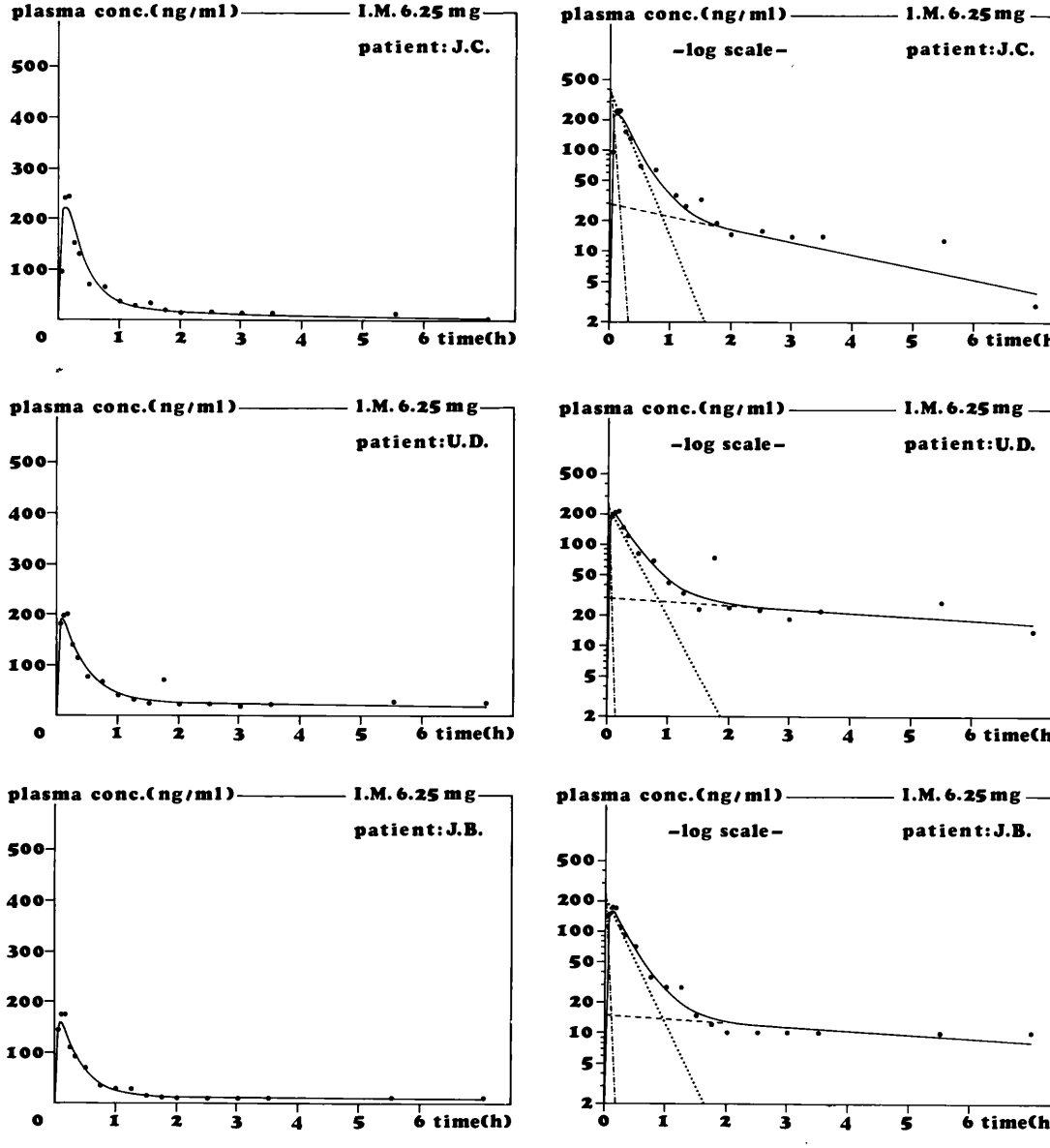
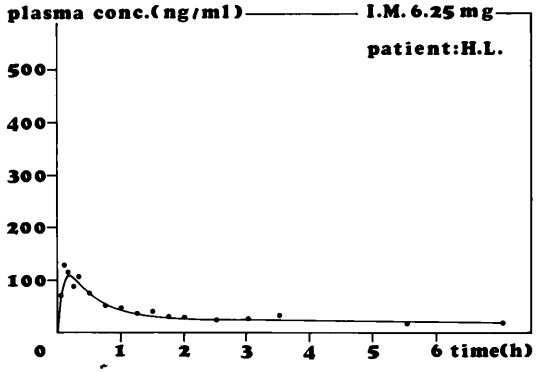
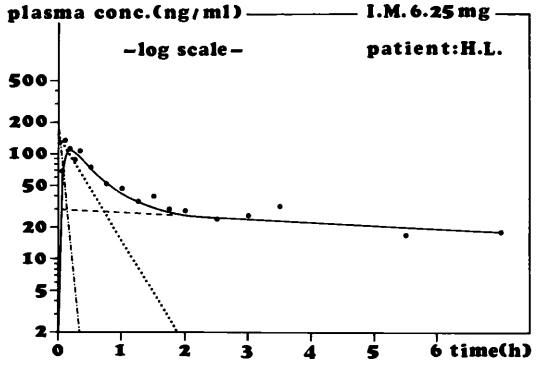
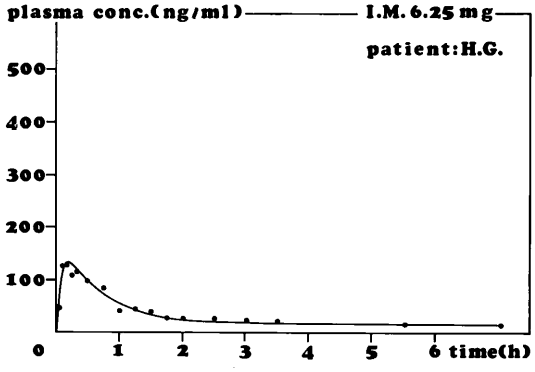
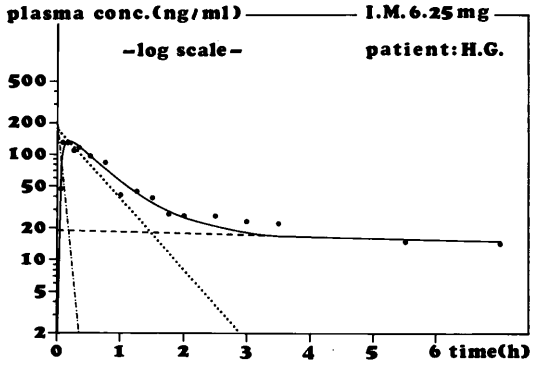


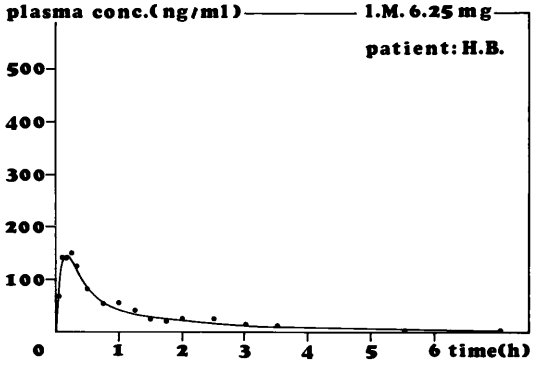
FIG. 1: *Plasma concentrations (ng/ml) after intramuscular injection of a dose of 6.25 mg. NAFFIT-1 programme. On the left the individual curves are given on linear scale; on the right side the curves are given in the same sequence but now on a semi-logarithmic scale.*

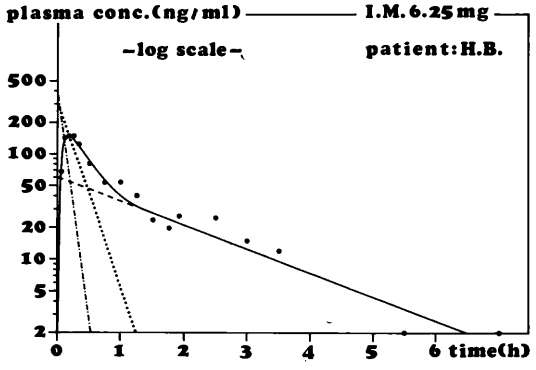

plasma conc.(ng/ml)
I.M. 6.25 mg
patient:H.L.
500
400
300
200
100
0
1
2
3
4
5
6 time(h)

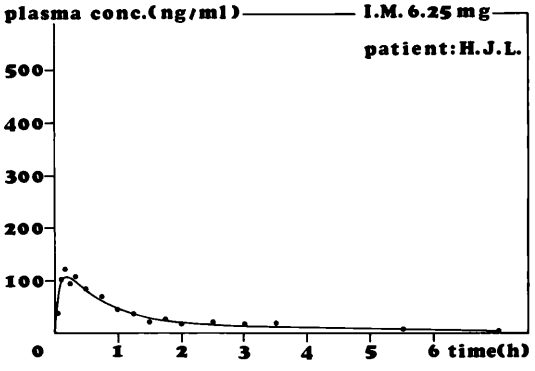

plasma conc.(ng/ml)
I.M. 6.25 mg
-log scale-
patient:H.L.
500
200
100
50
20
10
5
2
0
1
2
3
4
5
6 time(h)

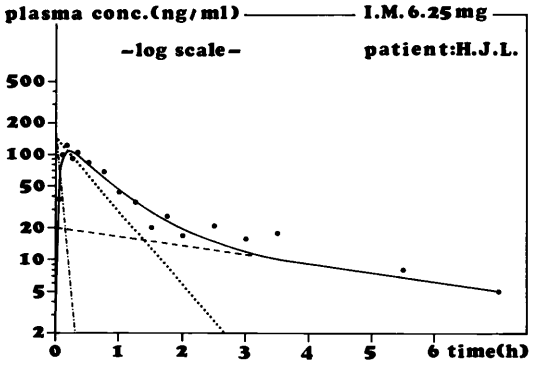

plasma conc.(ng/ml)
I.M. 6.25 mg
patient:H.G.
500
400
300
200
100
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.M. 6.25 mg
-log scale-
patient:H.G.
500
200
100
50
20
10
5
2
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.M. 6.25 mg
patient:H.B.
500
400
300
200
100
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.M. 6.25 mg
-log scale-
patient:H.B.
500
200
100
50
20
10
5
2
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.M. 6.25 mg
patient:H.J.L.
500
400
300
200
100
0
1
2
3
4
5
6 time(h)


plasma conc.(ng/ml)
I.M. 6.25 mg
-log scale-
patient:H.J.L.
500
200
100
50
20
10
5
2
0
1
2
3
4
5
6 time(h)

3.2.3.2. Determination of the bioavailability after oral administration
Fourteen patients with generalized obstructive lung diseases participated in this study. For reasons to be mentioned later (Chapters 9 and 10) four of them had to be excluded from the main group and were considered separately.
The resulting ten patients were aged between 18 en 62, mean 41 ± 18 (S.D.) years and weighed from 63 to 88 kg, mean 74 ± 8 (S.D.) kg. Four of these patients (A.O., K.W., A.V. and E.D.) also participated in the previously described experiment.
No electro-cardiograms were recorded.
All other conditions and methods were the same as described under 2.2 and 3.2.1.

3.2.3.3. The influence of muscle contraction on the bioavailability
Seven male volunteers participated in this study. They were aged between 23 and 29. The subjects were not suffering from any known illness.
In this study the subjects walked approximately 100 m during the first two minutes after injection and again for some two minute periods scattered over the experiments as indicated in Fig. 11. The same study was repeated without walking with an interval of three or more days. The experiments were terminated after 120 min.
No electro-cardiograms were recorded and urine was not collected.
All other conditions and methods were the same as described in Sections 2.2 and 3.2.1.

3.3. Results and discussion

3.3.1. Investigations with a dose of 6.25 mg

Determination of pharmacokinetic parameters using the NAFFIT-1 programme

The plasma concentration-time curves obtained in the seven patients are presented in Fig. 1 together with a representation on semi-logarithmic scale. The declining line of the semi-logarithmic plots, which relate thiazinamium methylsulphate concentration to time, was resolved into two exponential components.
The constants for the curve of the best fit were determined as described

earlier for the intravenous administration. In these calculations the general equation was:

$$c_p = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} - c_p^o \cdot e^{-k_a \cdot t} \qquad \text{(XIV)}$$

(for symbols see Appendix 1).

Pharmacokinetic parameters were determined (Table 1) on the assumption that all or most of the drug is eliminated in unchanged form. In absence of information to the contrary it was further assumed that drug elimination takes place exclusively in the central compartment. A representation of the model is given in Fig. 2. The equations used for calculation of the pharmacokinetic parameters are given in Appendix 1. It should be noted that in the equations for V_c and $(V_d)_\beta$ the factor D* should in fact be multiplied with F, the bioavailability of the drug. However, bioavailability after intramuscular administration was assumed to be 100% which results in F = 1 (which value is correct, as will be explained below).
In all seven patients absorption from the muscle tissue was found to be very rapid: the absorption half-life is 2.7 (mean) ± 1.0 (S.D.) min. This results in a peak in the plasma concentration-time curve between 6 and 10 min. after injection. The peak concentrations were found to be between 106 and 224 ng/ml with a mean value of 152 ± 43 (S.D.) ng/ml. This rapid absorption of this extremely water soluble compound is rather amazing

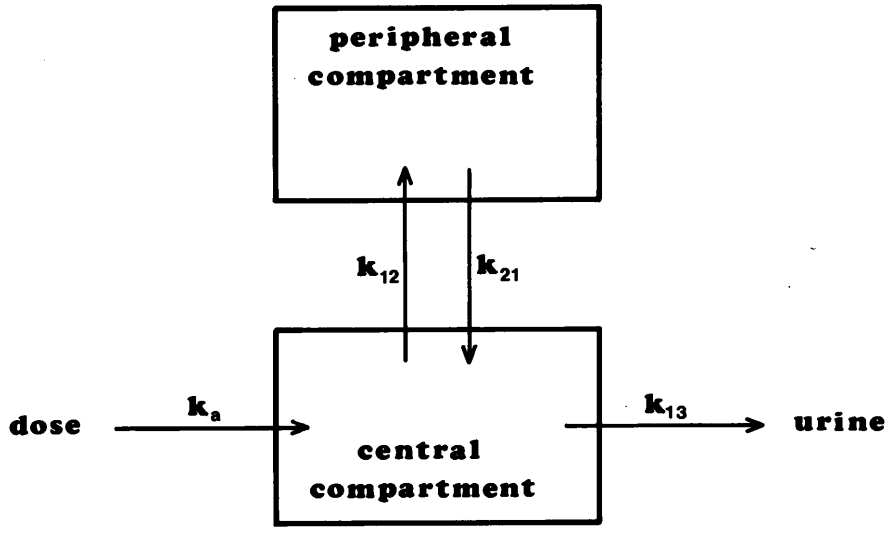


FIG. 2: *Schematic representation of an open two-compartment model with extravascular drug administration.*

TABLE I: *Pharmacokinetic parameters of thiazinamium methylsulphate after intramuscular injection of a dose of 6.25 mg in seven patients (NAFFIT-1 programme).*

Patient		J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	mean ± S.D.	averaged plasma conc. curve
Age	(year)	56	28	51	49	63	38	49	48 ± 12	
Body weight	(kg)	85	85	83	67	75	75	78	78 ± 7	
Height	(m)	1.78	1.80	1.75	1.76	1.78	1.65	1.78	1.78 ± 0.05	
Dose*	(mg)	8.11	8.11	8.11	8.11	8.11	8.11	8.11	8.11	
Dose*	(mg/kg)	0.0953	0.0953	0.0977	0.121	0.108	0.108	0.104	0.104 ± 0.009	
c_p^o	(ng/ml)	323	244	225	173	190	367	156	240 ± 79	221
k_a	(min.$^{-1}$)	0.28712	0.59073	0.46148	0.21424	0.22420	0.16953	0.20724	0.30779 ± 0.15762	0.32737
A	(ng/ml)	294	216	210	143	171	306	136	210 ± 68	187
α	(min.$^{-1}$)	0.04910	0.04132	0.04699	0.03812	0.02540	0.06774	0.02559	0.04204 ± 0.01471	0.04150
B	(ng/ml)	29	28	15	30	19	61	20	29 ± 15	34
β	(min.$^{-1}$)	0.00485	0.00124	0.00152	0.00125	0.00084	0.00869	0.00333	0.00310 ± 0.00285	0.00320
c_{max}	(ng/ml)	224	189	158	107	133	144	106	152 ± 43	151
t_{max}	(min.)	8	6	6	10	10	10	10	9 ± 2	8
$(t_{\frac{1}{2}})_a$	(min.)	2.4	1.2	1.5	3.2	3.1	4.1	3.3	2.7 ± 1.0	2.1
$(t_{\frac{1}{2}})_\alpha$	(min.)	12.6	16.8	14.8	18.2	26.8	10.2	27.1	18.1 ± 6.6	16.7
$(t_{\frac{1}{2}})_\beta$	(min.)	142.9	558.9	455.9	554.4	825.0	79.8	208.1	403.6 ± 270.3	216.6
k_{12}	(min.$^{-1}$)	0.02012	0.02795	0.02827	0.02549	0.01647	0.02611	0.00896	0.02191 ± 0.00716	0.02100
k_{21}	(min.$^{-1}$)	0.00882	0.00584	0.00455	0.00764	0.00330	0.01850	0.00618	0.00783 ± 0.00505	0.00909
k_{13}	(min.$^{-1}$)	0.02699	0.00887	0.01569	0.00623	0.00647	0.03181	0.01378	0.01569 ± 0.01010	0.01461
V_c	(l)	48.7	33.2	36.0	46.9	42.7	22.1	52.0	40.2 ± 10.5	36.7
V_c	(l/kg)	0.57	0.39	0.43	0.70	0.57	0.300	0.67	0.52 ± 0.15	
$(V_d)_\beta$	(l)	132.5	235.2	372.1	233.8	328.9	80.9	215.1	228.6 ± 101.6	167.5
$(V_d)_\beta$	(l/kg)	1.56	2.77	4.48	3.49	4.39	1.08	2.76	2.93 ± 1.30	
Cl_{tot}	(ml/min.)	1524.6	292.5	565.2	290.8	154.7	702.8	717.6	606.9 ± 459.8	535.8
r (correlation coefficient)		0.957	0.976	0.986	0.957	0.968	0.984	0.967		0.988

because it has generally been assumed that by contrast lipid soluble substances are absorbed more rapidly than insoluble ones (see Introduction). If the hypothesis is correct that water-soluble compounds can only be absorbed from the injection site by diffusion through water-filled intercellular pores in the capillary membrane (SCHOU, 1971), we must conclude that this diffusion is apparently very fast for thiazinamium cations. Although these pores cover only 0.2% of the total capillary surface, this does not seem to be a limiting factor. It must be assumed that the pores are of such size that thiazinamium cations, with a molecular weight of 299, can easily be transported through them (JONKMAN and others, 1976). These findings are in agreement with the statement of HOGBEN (1971) that "the typical capillary endothelium, such as that of the skeletal muscle capillary bed, is punctured by relatively large intercellular pores, that allow for free passage of most drugs or crystalloids up to the size of plasma proteins". So, good solubility of the drug in the interstitial fluid appeared to be of prime importance for rapid uptake.

As a result of this rapid uptake of the drug from the site of injection, the absorption process is completed within a short time, as is illustrated in Table 2. In this table the values for the absolute bioavailability (*i.e. versus* intravenous injection) of the intramuscular administration are given for the seven individual patients and the mean is calculated (see Chapter 1 of this Part). Because the plasma concentration-time curves had generally fallen to relatively low values within 120 min. the bioavailability was arbitrarily calculated for the time interval of 0 to 120 min. and 0 to 210 min. Still because a large number of samples were taken during this interval, the value of the bioavailability calculated can be considered to have adequate reliability.

As can be seen from the table the absolute bioavailability after intramuscular injection is essentially 100%. In one patient (J.C.) a rather high value was found, for which we can find no explanation.

The mean value for the seven patients was found to be 114% with a standard deviation of 20%. Comparison of the mean values for the area under the curve after intravenous and intramuscular administration by means of

TABLE 2: *The absolute bioavailability* (%) *after intramuscular injection of a dose of 6.25 mg.*

Patient	J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	mean ± S.D.	averaged plasma conc. curve
0–120 min.	149	98	104	125	129	100	93	114 ± 20	123
0–210 min.	144	113	96	143	130	97	96	117 ± 22	121

Student's t-test indicates that these values are not significantly different ($p > 0.05$). Discarding the value for J.C. the mean value becomes 108 ± 15 (S.D.)%. When the areas under the averaged data curves of the whole group after intramuscular and intravenous injection are compared, a bioavailability of 123% is found.

After the peak the plasma concentrations fall rapidly during the α-phase (= distribution phase). Comparison of the mean values for A and α, obtained after intravenous and intramuscular administration in the same group of patients shows that the A values are significantly different ($p < 0.01$) and also the α-values ($p < 0.001$) (for explanation see below; intravenous data are given in Chapter 2, Table 1).

We found for $(t_{\frac{1}{2}})_\alpha$ a mean value of 18.1 min. with a standard deviation of 6.6 min. These values are of the same magnitude as those obtained after intravenous administration but after intramuscular injection the $(t_{\frac{1}{2}})_\alpha$ value is generally somewhat longer.

It is interesting to note that from appr. 20 min. after the intramuscular injection the interindividual variations in the plasma concentrations are rather small (*e.g.* 25% at 45 min. after administration).

The apparent volumes of distribution of the central compartment for the individual patients vary from 22.1 to 52.0 l with a mean value of 40.2 ± 10.5 (S.D.) l. These values are again somewhat higher than those found after intravenous injection.

The distribution phase is followed by an elimination phase (β-phase). The mean values for B and β are not identical with those found after intravenous administration, but these differences proved to be not significant ($p > 0.05$).

The half-life $(t_{\frac{1}{2}})_\beta$ is much longer than the $(t_{\frac{1}{2}})_\alpha$. The individual values for $(t_{\frac{1}{2}})_\beta$ vary from 79.8 to 825.0 min. with a mean value of 403.6 min. (standard deviation 270.3 min.). It should be realized, however, that this mean value has been greatly influenced by the extremely high value for patient H.G. and by the low value for patient H.B. If these two data are discarded a mean value of 384.0 (standard deviation 196.1) is obtained. Just as was the case for the values of $(t_{\frac{1}{2}})_\alpha$, the $(t_{\frac{1}{2}})_\beta$ likewise appears to be longer after intramuscular administration than after intravenous administration.

A possible explanation for these higher values after intramuscular injection may be that the absorption process from the muscle is not monophasic (as was assumed in the NAFFIT-1 calculations) but biphasic. This explanation rests on the assumption that the rapid first absorption phase is followed by a slower second one (*N.B.* The autoradiographic study on intramuscular injection in mice gives some indication of such a phenomenon (see Chapter 13).

SUND and SCHOU (1964) reported that when radioactive neutral - water soluble - compounds or ions are used in absorption studies, it was observed that plots of the logarithm of the fraction of drug remaining at the site of

injection *versus* time may not be linear. For *e.g.* sucrose, mannitol and inulin the absorption half-life at the beginning of the experiment is often shorter than that calculated later on.
It will be clear from Fig. 1 that most plasma concentrations in the β-phase are very low and fall to zero or nearly zero at the end of the experiment. Moreover, in certain instances there are some fluctuations in the plasma concentrations, which leads to a rather high standard deviation. These two factors cause the reliability of the pharmacokinetic parameters derived to be limited (see below).
The apparent volumes of distribution of the β-phase (the peripheral compartment) vary from 80.9 to 372.1 l (mean 228.6 ± 101.6 (S.D.) l).
The high values for the rate constants for absorption k_a reflect the rapid uptake from the muscle. This process seems to be appr. 7 to 23 (mean 14) times faster than the transfer from the central to the peripheral compartment k_{12}. On the other hand, the values for k_{12} are appr. 2 to 6 (mean 3) times greater than those for k_{21}. The rate constants for the elimination from the central compartment k_{13} are of the same order of magnitude as found for the transfer between the central compartment and the peripheral compartment.
The total clearance for thiazinamium methylsulphate was found to be high: it varied from 154.7 to 1524.6 ml/min., with a mean value of 606.9 ± 459.8 (S.D.) ml/min. This value is in fair agreement with the findings after intravenous injection although there are individual variations, partly due to the factors described above.
For some pharmacokinetic parameters it is another interesting matter to compare the mean values calculated from the values for the individual patients (Table 1) with the values for the same parameters calculated from the curve that was constructed from the averaged plasma concentrations (Fig. 3).
In the former case we found: $(t_{\frac{1}{2}})_\alpha$ = 18.1 min., $(t_{\frac{1}{2}})_\beta$ = 403.6 min., V_c = 40.2 l and $(V_d)_\beta$ = 228.6 l.
In the latter case we found: $(t_{\frac{1}{2}})_\alpha$ = 16.7 min., $(t_{\frac{1}{2}})_\beta$ = 216.6 min., V_c = 36.7 l and $(V_d)_\beta$ = 167.5 l. From these data it may be concluded that there is a good correlation for the values of the α-phase, but there is a distinct difference for the β-phase values.

Comparison with other studies on thiazinamium methylsulphate after intramuscular injection is difficult.
KERCKHOFFS and HUIZINGA (1967) published some results of a similar study, but as already mentioned in Part II, Section 2.1. their approach to the analysis of the drug was possibly incorrect and hence the results are questionable.
The results of our study are in good agreement with the information on other quaternary ammonium compounds available from literature when administered intramuscularly to man.

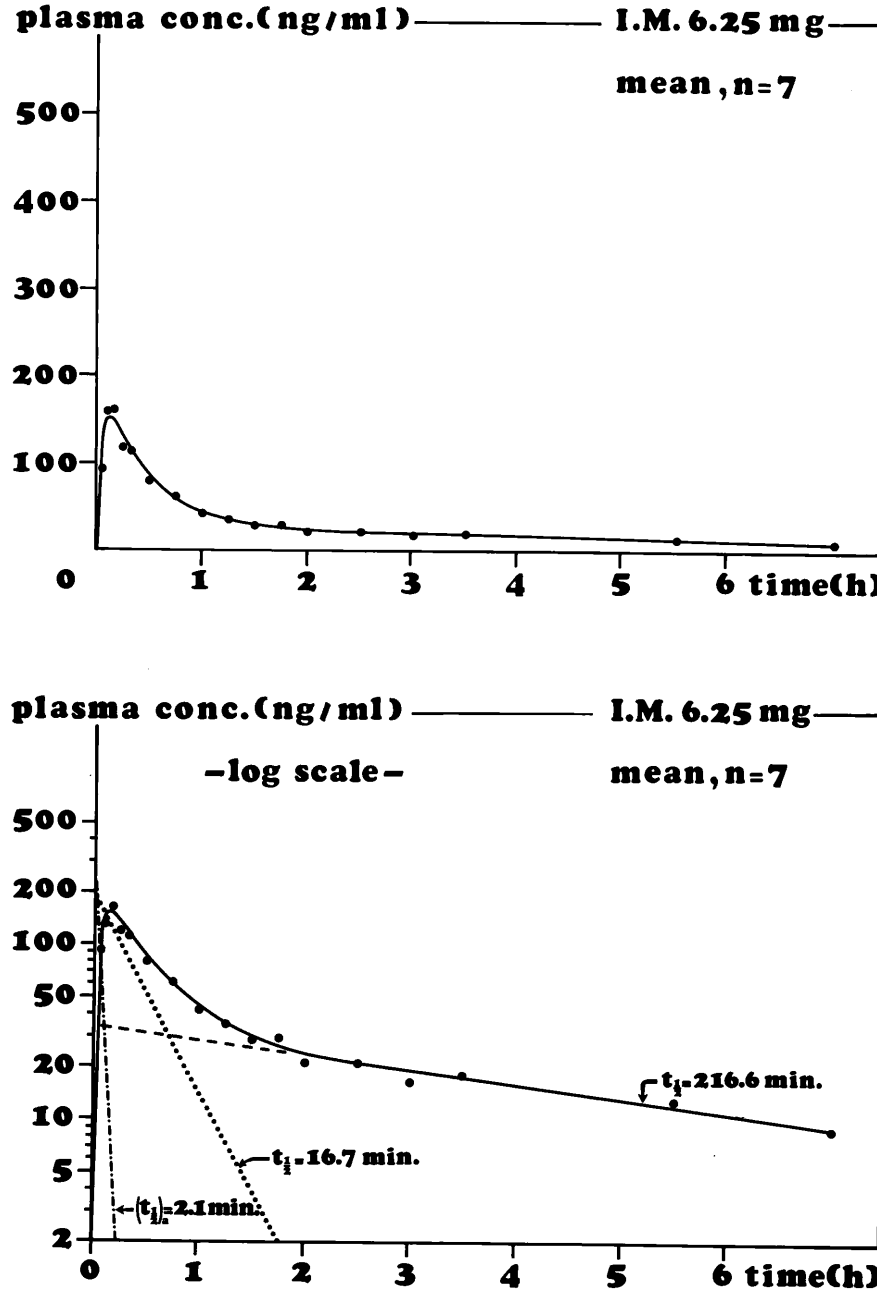


FIG. 3: *Plasma concentrations (ng/ml) after intramuscular injection of a dose of 6.25 mg. NAFFIT-1 programme. The curve has been constructed from the averaged plasma concentrations of the seven patients. Above on linear scale, below on semi-logarithmic scale.*

SUNDWALL (1960) reported a very rapid absorption also for pralidoxime (as methylsulphate).
SIDELL and GROFF (1971) also reported a rapid uptake from the muscle for the same drug (given as chloride). Already 5 min. after injection the levels were more than 50% of the peak plasma concentrations which were obtained after approximately 15 min. A biological half-life $(t_{\frac{1}{2}})_{\beta}$ of 77 min. was reported. No other pharmacokinetic parameters were reported.
SUNDWALL and others (1973) gave plasma concentration-time curves, for emepronium bromide (Cetiprin®). Maximum plasma concentrations were found after appr. 15 min., which also suggests rapid absorption. The biological half-lives ranged from 90 tot 135 min., which is a little shorter, but in the same order of size as we found for thiazinamium methylsulphate.
Rapid onset of pharmacological action was reported by MÖLLER and ROSÉN (1968) for propantheline bromide, methylscopolamine nitrate and butylscopolamine bromide; this also suggested rapid uptake of the drugs from the muscle (maximum effect after 15 to 30 min.).
Absence of comparison of the area under the plasma concentration-time curve with that obtained after intravenous administration, however, does not allow bioavailability calculation in all above mentioned studies.
For all we know, no papers have appeared which deal with a sophisticated pharmacokinetic evaluation of plasma concentration data, found after intramuscular administration of quaternary ammonium compounds in man.

Determination of pharmacokinetic parameters using the NONLIN programme

Using the information obtained from the calculations with the data of the experiment with intravenous administration, we decided to use again weighting factors of $W = {}^{1}/\sqrt{y_i}$ for all observations. This results in an improvement of the fit as compared to that obtained with a single weighting factor of $W = 1$.
Moreover, we decided to investigate if a better fit could be achieved by introducing a lag-time in the equation used. This, because in spite of the fact that with the NAFFIT-1 calculations a very rapid absorption was found, theoretically it could not be excluded that after intramuscular injection some time passes before the absorption process starts (*N.B.* In the NAFFIT-1 programme no lag-time is included).
In this case, the equation to be used in the calculation is:

$$c_p = A \cdot e^{-\alpha(t-t_0)} + B \cdot e^{-\beta(t-t_0)} - c_p^o \cdot e^{-k_a(t-t_0)} \qquad \text{(XV)}$$

(for symbols see Appendix 1).

TABLE 3: *Pharmacokinetic parameters of thiazinamium methylsulphate after intramuscular injection of a dose of 6.25 mg in seven patients. NONLIN programme. Weighting factor* $W = {}^{1}/\sqrt{y_i}$.

Patient		J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	mean ± S.D.	averaged plasma conc. curve
c_p^o	(ng/ml)	420	312	229	138	153	290	144	241 ± 106	199
t_o	(min.)	2.3	0.1	1.4	2.6	2.4	1.4	2.2	1.8 ± 0.9	2.0
k_a	(min.$^{-1}$)	0.47631	0.42246	0.77966	1.5672	0.61072	0.23988	0.39760	0.64198 ± 0.44216	0.66428
A	(ng/ml)	369	274	214	105	131	226	122	206 ± 95	167
α	(min.$^{-1}$)	0.08699	0.05997	0.05129	0.03291	0.02365	0.06375	0.02672	0.04933 ± 0.02303	0.04120
B	(ng/ml)	52	38	15	33	22	64	22	35 ± 18	32
β	(min.$^{-1}$)	0.00741	0.00228	0.00140	0.00149	0.00095	0.00848	0.00292	0.00356 ± 0.00308	0.00299
c_{max}	(ng/ml)	246	204	177	125	130	153	115	164 ± 48	160
t_{max}	(min.)	6	6	6	6	10	10	10	8 ± 2	6
$(t_{\frac{1}{2}})_a$	(min.)	1.5	1.6	0.9	0.4	1.1	2.9	1.7	1.4 ± 0.8	1.0
$(t_{\frac{1}{2}})_\alpha$	(min.)	8.0	11.6	13.5	21.1	29.3	10.9	25.9	17.2 ± 8.2	16.8
$(t_{\frac{1}{2}})_\beta$	(min.)	93.5	303.9	495.0	465.1	729.5	81.7	237.3	343.7 ± 234.7	231.8
k_{12}	(min.$^{-1}$)	0.03986	0.03825	0.03264	0.01995	0.01505	0.02541	0.01118	0.02605 ± 0.01127	0.02157
k_{21}	(min.$^{-1}$)	0.01728	0.00931	0.00467	0.00900	0.00421	0.02068	0.00656	0.01024 ± 0.00635	0.00913
k_{13}	(min.$^{-1}$)	0.03730	0.01469	0.01538	0.00545	0.00533	0.02614	0.01190	0.01660 ± 0.01153	0.01349
V_c	(l)	19.3	26.0	35.4	58.8	53.0	28.0	56.3	39.5 ± 16.2	40.8
V_c	(l/kg)	0.23	0.31	0.43	0.88	0.71	0.37	0.72	0.52 ± 0.25	
$(V_d)_\beta$	(l)	97.2	167.5	389.1	214.8	297.5	86.2	229.5	211.7 ± 108.0	183.8
$(V_d)_\beta$	(l/kg)	1.14	1.97	4.69	3.21	3.97	1.15	2.94	2.72 ± 1.37	
Cl_{tot}	(ml/min.)	719.9	381.9	547.5	320.5	282.5	731.9	670.0	522.0 ± 192.9	550.4
r (correlation coefficient)		0.994	0.984	0.991	0.987	0.989	0.993	0.989		0.997

The results of these calculations (see Table 3) indicate that indeed a better fit is obtained by the latter method suggesting that a lag-time occurs, although, it was very short (between 0.1 and 2.6 min.). The existence of a lag-time in the NONLIN calculations results in an increase of the k_a value. As a consequence the mean value for k_a now differs significantly (Student's t-test; $p < 0.01$) from the mean value obtained by NAFFIT-1 calculations. If we compare the mean values for A, α, B and β no significant difference could be found ($p > 0.05$).
A typical example of a plasma concentration-time curve obtained by calculation with the NONLIN programme ($W = 1/\sqrt{y_i}$ and a lag-time included) is represented in Fig. 4.

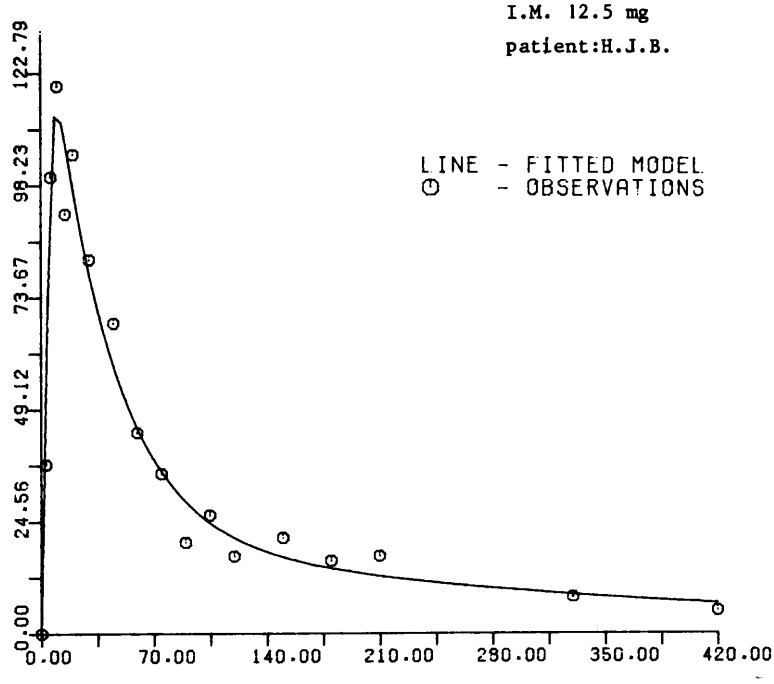


FIG. 4: *An example of a direct computer plot of a plasma concentration-time curve obtained after intramuscular injection of a dose of 6.25 mg. NONLIN programme (Weighting factor* $W = {}^{1}/\sqrt{y_i}$*).*

In Fig. 5 and Fig. 6 the results of the calculations of NAFFIT-1 and NONLIN for the line of the best fit are given for patient H.G. and the mean data, respectively.

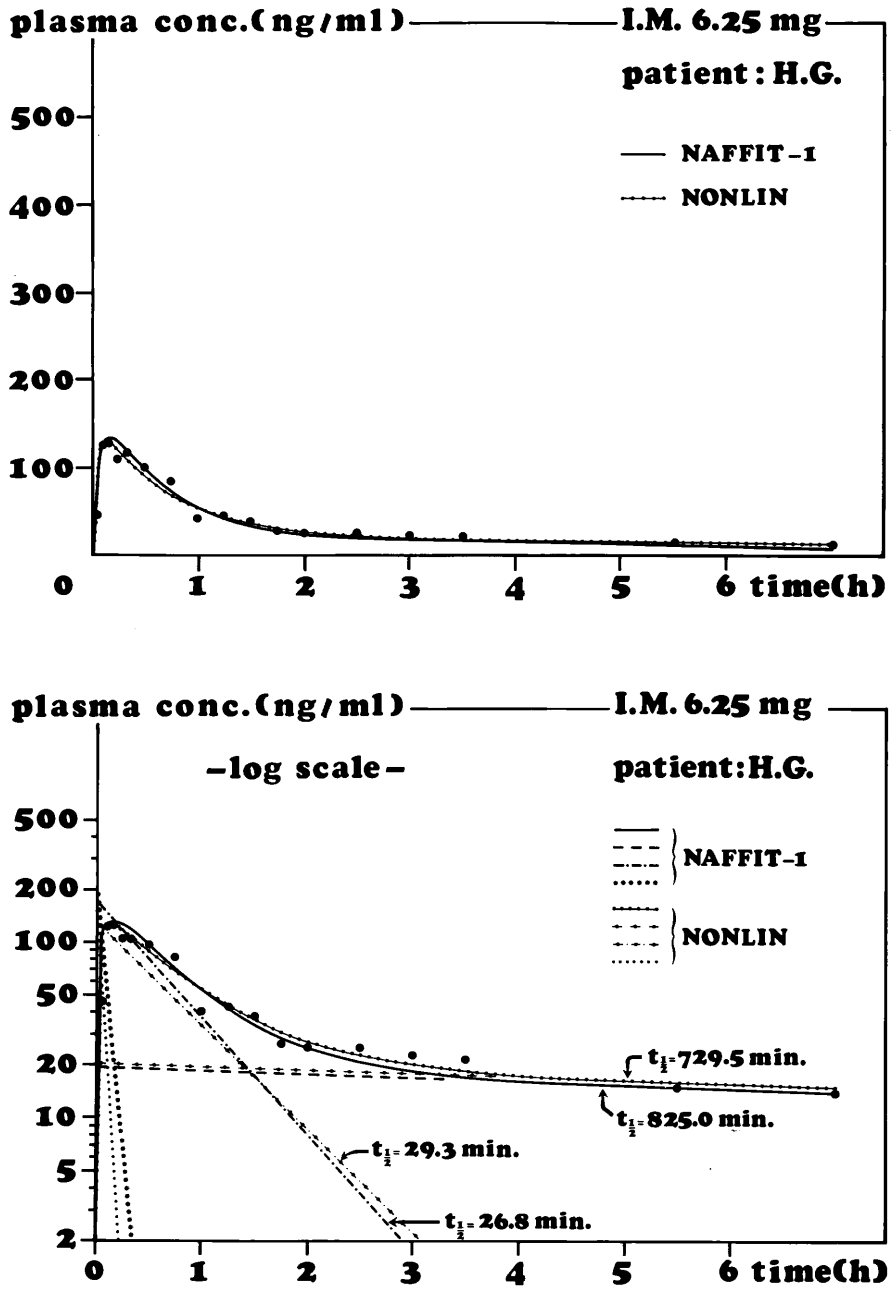


FIG. 5: *Plasma concentrations of patient H.G. after intramuscular injection of a dose of 6.25 mg. The curve of the best fit has been calculated both by using the NAFFIT-1 and NONLIN programme. Using the latter a weighting factor of* $W = {}^{1}/\sqrt{y_i}$ *was applied. The above picture represents the curves on linear scale; below the curves on semi-logarithmic scale are given.*

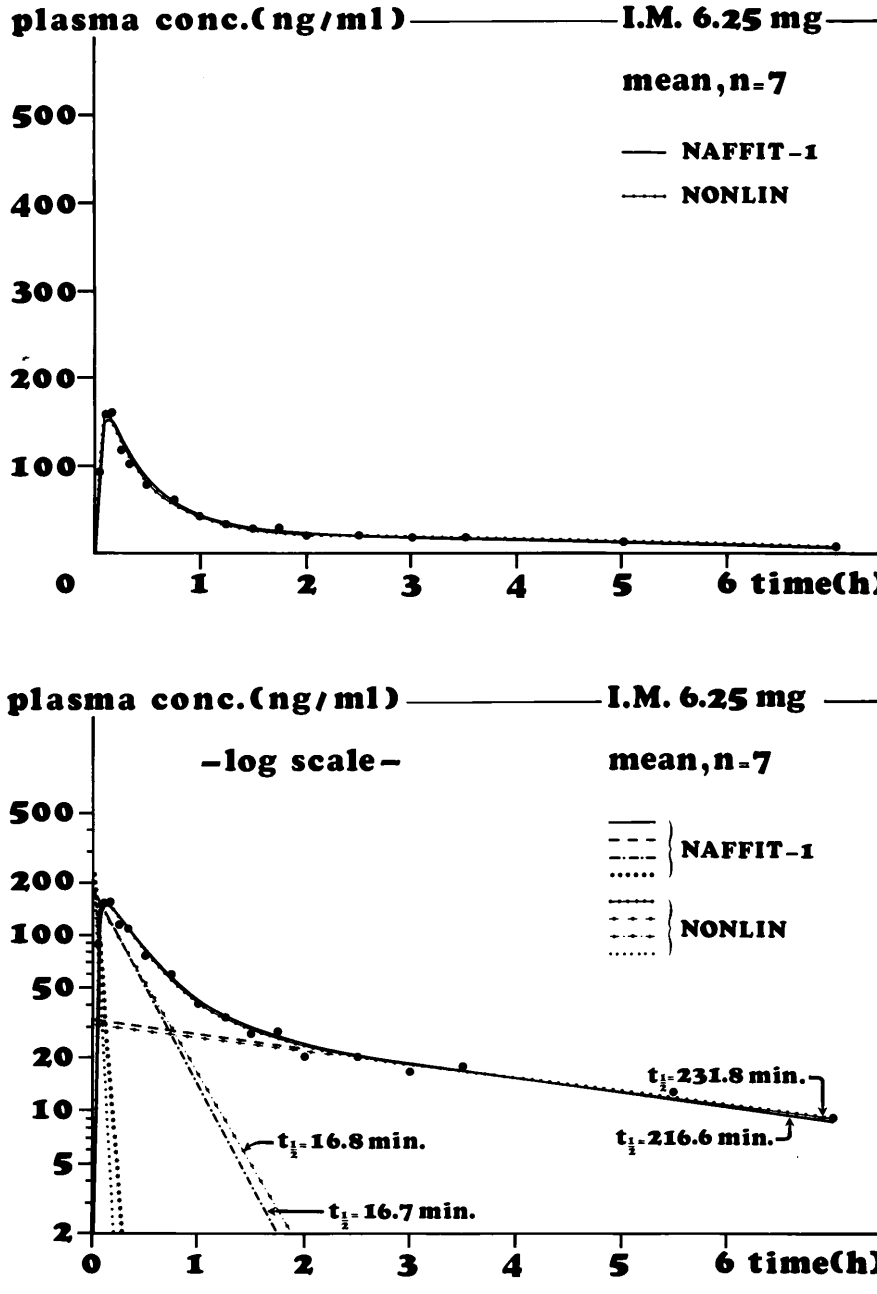


FIG. 6: *The curve of the averaged plasma concentrations of seven patients after intramuscular injection of a dose of 6.25 mg. The curve of the best fit has been calculated both by using the NAFFIT-1 and NONLIN programme. Using the latter a weighting factor of* $W = 1/\sqrt{y_i}$ *was applied. The above picture represents the curves on linear scale; below the curves on semi-logarithmic scale are given.*

TABLE 4: *Pharmacokinetic parameters of thiazinamium methylsulphate after intramuscular injection of a dose of 12.5 mg in seven patients (NAFFIT - 1 programme).*

Patient		A.O.	K.W.	A.V.	E.D.	N.S.	E.W.	E.P.	mean ± S.D.	averaged plasma conc. curve
Age	(year)	62	22	29	54	22	43	48	40 ± 16	
Body weight	(kg)	82	68	71	69	80	81	82	76 ± 6	
Height	(m)	1.67	1.90	1.80	1.67	1.86	1.78	1.78	1.78 ± 0.09	
Dose*	(mg)	16.22	16.22	16.22	16.22	16.22	16.22	16.22	16.22	
Dose*	(mg/kg)	0.1978	0.2385	0.2285	0.2351	0.2028	0.2002	0.1978	0.2144 ± 0.0187	
c_p^o	(ng/ml)	347	747	489	529	345	437	385	468 ± 141	477
k_a	($min.^{-1}$)	0.22267	0.25343	0.32753	0.69376	0.22317	0.65114	0.23862	0.37291 ± 0.20806	0.30499
A	(ng/ml)	284	704	426	478	278	357	307	405 ± 152	414
α	($min.^{-1}$)	0.02986	0.05545	0.04740	0.04693	0.04263	0.03894	0.02990	0.04159 ± 0.00946	0.04665
B	(ng/ml)	63	43	63	51	67	80	78	64 ± 13	63
β	($min.^{-1}$)	0.00326	0.00103	0.00168	0.00171	0.00729	0.00298	0.00369	0.00309 ± 0.00209	0.00265
c_{max}	(ng/ml)	225	396	318	403	207	352	267	311 ± 77	305
t_{max}	(min.)	12	8	8	6	10	6	12	9 ± 3	8
$(t_{\frac{1}{2}})_a$	(min.)	3.1	2.7	2.1	1.0	3.1	1.1	2.9	2.3 ± 0.9	2.3
$(t_{\frac{1}{2}})_\alpha$	(min.)	23.2	12.5	14.6	14.8	16.3	17.8	23.2	17.5 ± 4.2	14.9
$(t_{\frac{1}{2}})_\beta$	(min.)	212.6	672.8	412.5	405.3	95.1	232.6	187.8	316.9 ± 194.7	261.5
k_{12}	($min.^{-1}$)	0.01300	0.03860	0.03099	0.02935	0.01381	0.02022	0.01233	0.02261 ± 0.01043	0.02623
k_{21}	($min.^{-1}$)	0.00809	0.00416	0.00757	0.00607	0.01415	0.00956	0.00900	0.00837 ± 0.00313	0.00846
k_{13}	($min.^{-1}$)	0.01203	0.01372	0.01052	0.01323	0.02196	0.01213	0.01226	0.01369 ± 0.00378	0.01461
V_c	(l)	46.7	21.7	33.2	30.7	47.0	37.1	42.1	36.9 ± 9.2	34.0
V_c	(l/kg)	0.57	0.32	0.47	0.44	0.59	0.46	0.51	0.48 ± 0.09	
$(V_d)_\beta$	(l)	172.5	289.3	207.7	237.1	141.6	151.1	140.0	191.3 ± 56.3	187.5
$(V_d)_\beta$	(l/kg)	2.1	4.3	2.9	3.4	1.8	1.9	1.7	2.6 ± 1.0	
Cl_{tot}	(ml/min.)	562.3	297.9	349.0	405.6	1032.3	450.0	516.5	516.2 ± 245.2	496.8
r (correlation coefficient)		0.949	0.968	0.960	0.984	0.988	0.992	0.954		0.992

3.3.2. Investigations with a dose of 12.5 mg

3.3.2.1. Comparison with a dose of 25.0 mg

The pharmacokinetic parameters obtained in this study by employment of the NAFFIT-1 programme are given in Table 4 (for equations and model see Section 3.3.1.).

Although different groups of patients are involved, it is interesting to compare the data of Table 4 with those of Table 1, which contains the results of the experiment with the 6.25 mg dose. If one compares the mean values a striking similarity is seen. The absorption rate constant is slightly higher after a dose of 12.5 mg, but in both experiments the maximum plasma concentration is reached after 8 min. With the 12.5 mg dose the peak is twice as high as with the 6.25 mg dose. The values for α and β are almost the same in both experiments, and in the 12.5 mg experiment the values for A and B are double those found in the 6.25 mg experiment. As a consequence $(t_{\frac{1}{2}})_\alpha$ and $(t_{\frac{1}{2}})_\beta$ show a good resemblance. The rate constants k_{12}, k_{21} and k_{13}, the volumes of distribution and total plasma clearance, are also quite similar. The magnitude of all these data and what they mean has been discussed in 3.3.1.

Examples of plasma concentration-time curves are given in Fig. 7 and Fig. 8 (see 3.3.3.1). A more detailed discussion of the comparison of the 12.5 mg and 25.0 mg doses in the same patients will also be given there.

3.3.2.2. Determination of the bioavailability after rectal administration

The aim of this study was to create an opportunity for calculating the bioavailability after rectal administration (see Part III, Chapter 5). To this purpose the areas under the plasma concentration-time curves were used. No other pharmacokinetic parameters were calculated from the results of this experiment and it is here mentioned only for the sake of completeness.

3.3.3. Investigations with a dose of 25.0 mg

3.3.3.1. Comparison with a dose of 12.5 mg

Determination of pharmacokinetic parameters using the NAFFIT-1 programme

The pharmacokinetic parameters obtained in this study by employment of the NAFFIT-1 programme are given in Table 6 (for equations and model see Section 3.3.1.). Examples of plasma concentration-time curves for both doses are given in Fig. 7 (patient E.P.) and Fig. 8 (averaged data curve). Comparison of the data and curves obtained with the 12.5 and 25.0 mg doses demonstrate that several parameters obtained with the higher dose

TABLE 6: *Pharmacokinetic parameters of thiazinamium methylsulphate after intramuscular injection of a dose of 25.0 mg in seven patients (NAFFIT-1 programme).*

Patient		A.O.	K.W.	A.V.	E.D.	N.S.	E.W.	E.P.	mean ± S.D.	averaged plasma conc. curve
Age	(year)	62	22	29	54	22	43	48	40 ± 16	
Body weight	(kg)	82	68	71	69	80	81	82	76 ± 6	
Height	(m)	1.67	1.90	1.80	1.67	1.86	1.78	1.78	1.78 ± 0.09	
Dose*	(mg)	32.44	32.44	32.44	32.44	32.44	32.44	32.44	32.44	
Dose*	(mg/kg)	0.3956	0.4771	0.4569	0.4701	0.4055	0.4005	0.3956	0.4288 ± 0.0374	
c_p^o	(ng/ml)	514	891	371	483	357	907	487	573 ± 231	564
k_a	(min.$^{-1}$)	0.50086	0.57296	0.40509	0.41790	0.29761	0.35342	0.38068	0.41836 ± 0.09229	0.37581
A	(ng/ml)	440	792	314	441	316	849	393	506 ± 221	476
α	(min.$^{-1}$)	0.03107	0.03572	0.01717	0.01933	0.02031	0.03455	0.02660	0.02639 ± 0.00761	0.02899
B	(ng/ml)	74	99	57	42	41	58	94	66 ± 23	88
β	(min.$^{-1}$)	0.00211	0.00275	0.00116	0.00039	0.00117	0.00165	0.00155	0.00154 ± 0.00075	0.00272
c_{max}	(ng/ml)	413	708	314	403	280	648	387	450 ± 164	436
t_{max}	(min.)	6	6	8	8	10	8	8	8 ± 1	8
$(t_{\frac{1}{2}})_a$	(min.)	1.4	1.2	1.7	1.7	2.3	2.0	1.8	1.7 ± 0.4	1.8
$(t_{\frac{1}{2}})_\alpha$	(min.)	22.3	19.4	40.4	35.9	34.1	20.1	26.1	28.3 ± 8.4	23.9
$(t_{\frac{1}{2}})_\beta$	(min.$^{-1}$)	328.4	252.0	597.4	1776.9	592.3	420.0	447.1	630.6 ± 521.1	254.8
k_{12}	(min.$^{-1}$)	0.01646	0.01674	0.00921	0.01398	0.01106	0.01726	0.01531	0.01429 ± 0.00308	0.01333
k_{21}	(min.$^{-1}$)	0.00628	0.00641	0.00362	0.00204	0.00337	0.00375	0.00639	0.00455 ± 0.00178	0.00682
k_{13}	(min.$^{-1}$)	0.01044	0.01532	0.00550	0.00370	0.00706	0.01519	0.00646	0.00910 ± 0.00467	0.01156
V_c	(l)	63.1	36.4	87.4	67.2	90.9	35.8	66.6	63.9 ± 21.8	58.3
V_c	(l/kg)	0.77	0.54	1.23	0.97	1.14	0.44	0.81	0.84 ± 0.29	
$(V_d)_\beta$	(l)	312.3	202.8	414.8	637.4	547.9	329.2	277.5	388.8 ± 155.0	244.5
$(V_d)_\beta$	(l/kg)	3.81	2.98	5.84	9.24	6.85	4.06	3.38	5.17 ± 2.27	
Cl_{tot}	(ml/min.)	658.8	557.7	480.7	248.6	641.8	543.8	430.2	508.8 ± 140.5	674.0
r (= correlation coefficient)		0.987	0.996	0.961	0.938	0.935	0.970	0.985		0.998

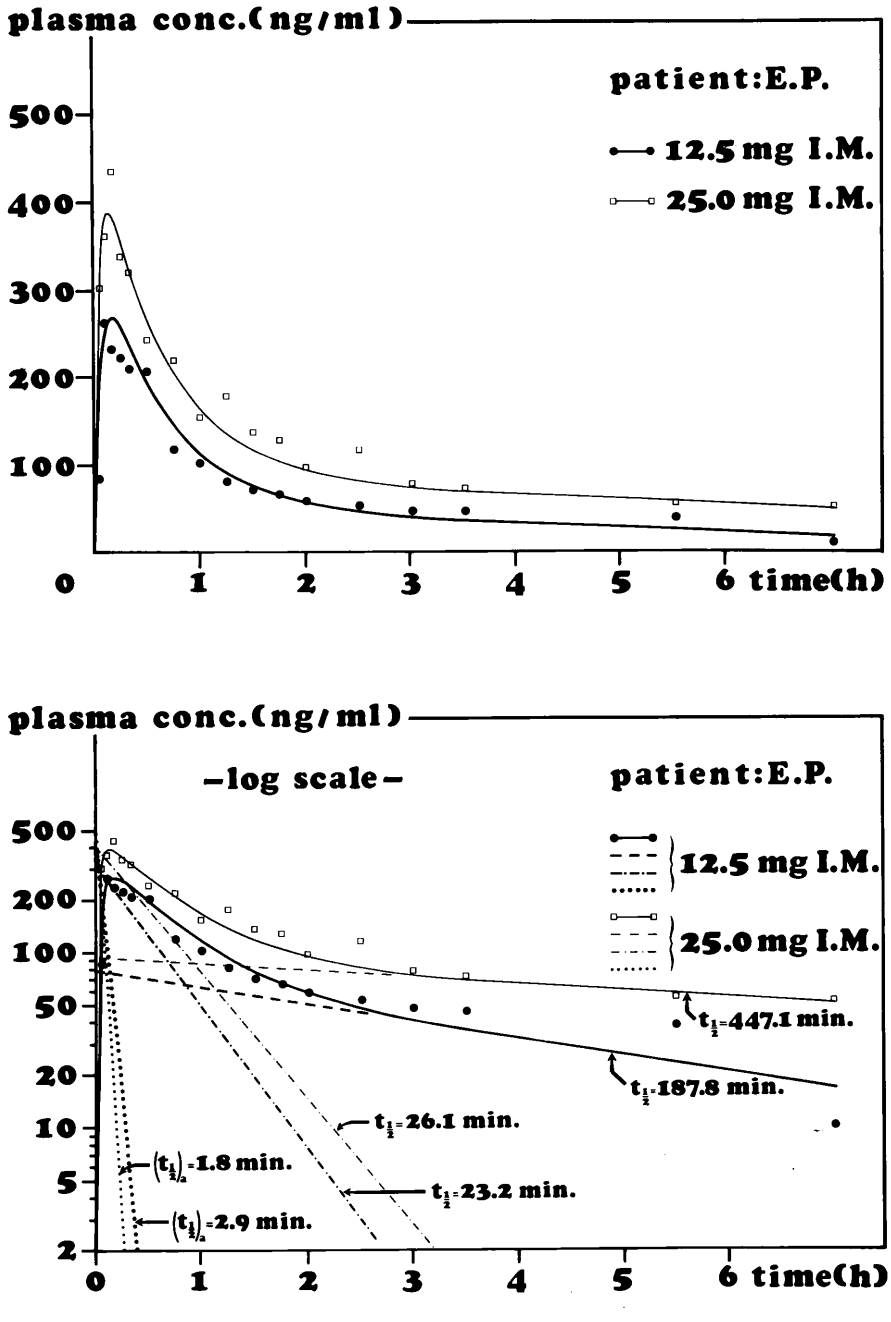


FIG. 7: *Plasma concentration-time curve after intramuscular injection of a dose of 12.5 and 25.0 mg. NAFFIT-1 programme. Patient: E. P. Above on linear scale, below on semi-logarithmic scale.*

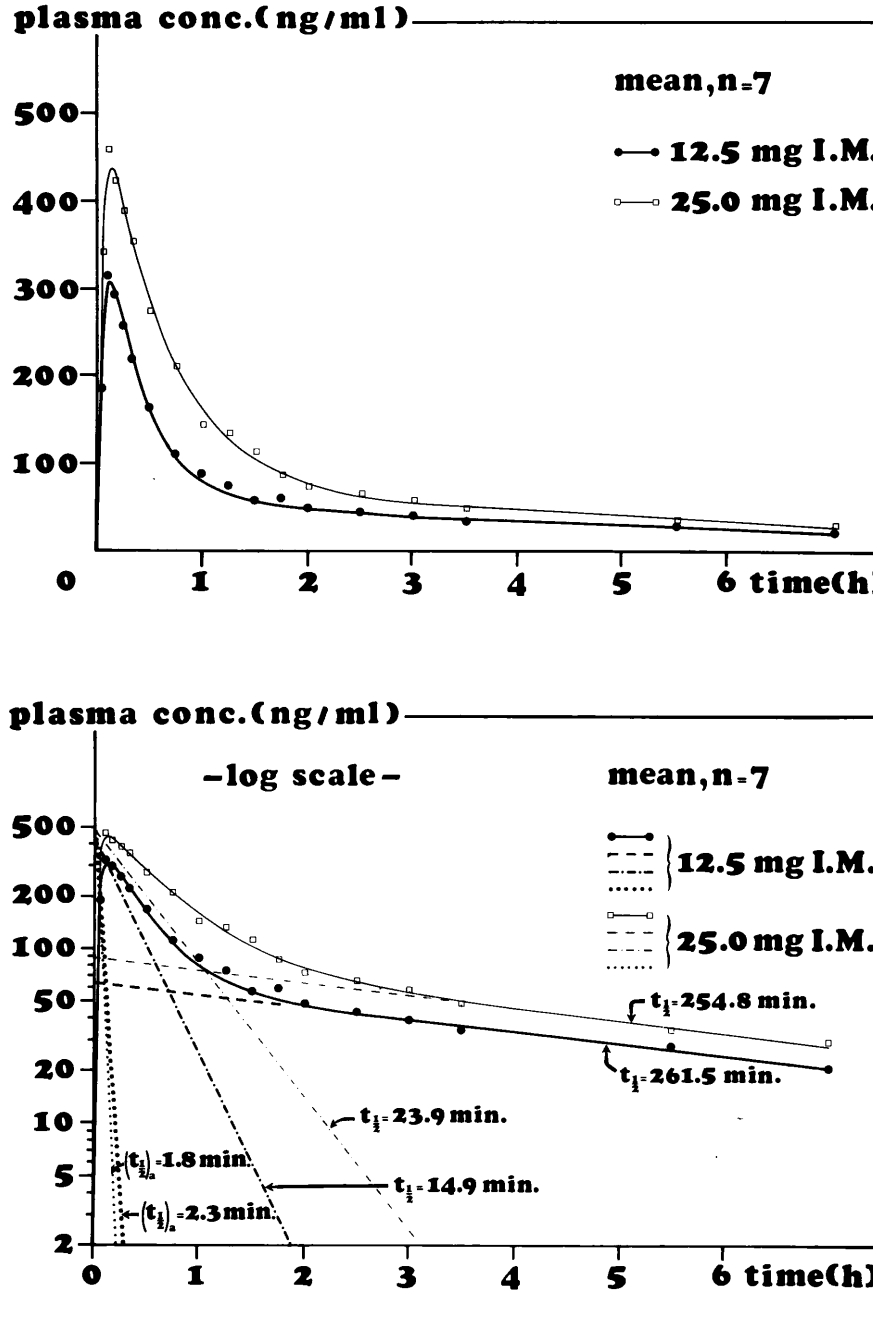


FIG. 8: *Plasma concentration-time curve after intramuscular injection of a dose of 12.5 and 25.0 mg. NAFFIT-1 programme. Averaged data curve (n = 7). Above on linear scale, below on semi-logarithmic scale.*

are not double those obtained with the lower dose as was expected, *e.g.* c_p^o, A, B and c_{max}. For the mean values of A and B no significant difference could be found (Student's t-test, $p > 0.05$). The values for c_{max} vary from 280 to 708 ng/ml (mean 450 ± 164 (S.D.) ng/ml). In the patient (K.W.) with the highest value for k_a, the value for c_{max} was found to be the highest. The peak was achieved after 6 min., whereas it was achieved after 10 min. in the patient (N.S.) with the lowest values for k_a and c_{max}. In one patient (E.D.) c_{max} was exactly the same after injection of 12.5 and 25.0 mg. Although the rate constants for the absorption process were almost identical in both experiments (with $(t_{\frac{1}{2}})_a$ of appr. 2 min.), the constants for distribution (α) and the overall rate constants for elimination (β) of the drug were both considerably lower in the 25.0 mg experiment. The difference for the mean value of α was found to be significant ($p < 0.01$) but the difference for the mean value of β was not ($p > 0.05$). So, both $(t_{\frac{1}{2}})_\alpha$ and $(t_{\frac{1}{2}})_\beta$ are substantially larger in most of the patients. The volumes of distribution for both the central and the peripheral compartment are also larger in the experiment with the higher dose. Theoretically, the increase of $(t_{\frac{1}{2}})_\alpha$ and $(t_{\frac{1}{2}})_\beta$ with increasing dose could have been caused by limited biotransformation or elimination, but the fact that total clearance was not found to be considerably lower (in some patients even higher) in the 25.0 mg experiment contradicts this hypothesis. It is possible that the explanation given in Section 3.3.1 applies, namely that the absorption process is biphasic. In this respect the findings of SCHOU (1971) are important, who reported that anticholinergic drugs inhibit their own absorption from the muscle by causing local vasoconstriction and hence diminishing the capillary blood flow. This inhibition seems to increase with concentration. Comparison of the area under the plasma concentration-time curves after the two doses of thiazinamium methylsulphate also leads to the conclusion that higher doses of the drug may diminish their own absorption. Table 7 shows that, during the time of the experiment, the area under the plasma concentration-time

TABLE 7: *The area under the plasma concentration-time curve (A.U.C.) after intramuscular injection of a dose of 25.0 mg expressed as percentage of the A.U.C. after a dose of 12.5 mg in the same patient.*

Patient	A.O.	K.W.	A.V.	E.D.	N.S.	E.W.	E.P.	mean ± S.D.	averaged plasma conc. curve
0–120 min.	145	208	153	168	178	168	138	165 ± 23	170
0–210 min.	144	203	150	167	185	153	143	164 ± 23	167
0–420 min.	136	185	139	159	209	141	161	161 ± 27	160

TABLE 5: *Pharmacokinetic parameters of thiazinamium methylsulphate after intramuscular injection of a dose of 12.5 mg in seven patients. NONLIN programme. Weighting factor* $W = {}^{1}/\sqrt{y_i}$.

Patient		A.O.	K.W.	A.V.	E.D.	N.S.	E.W.	E.P.	mean ± S.D.	averaged plasma conc. curve
c_p^o	(ng/ml)	322	470	401	479	802	475	291	463 ± 168	387
t_o	(min.)	2.5	2.8	2.4	2.3	0.7	0.5	2.6	2.0 ± 1.0	1.9
k_a	(min.$^{-1}$)	0.40942	1.8612	0.72184	2.7653	0.15232	0.63889	1.0153	1.0806 ± 0.9209	0.67852
A	(ng/ml)	237	430	326	437	727	367	213	391 ± 172	322
α	(min.$^{-1}$)	0.03392	0.04198	0.05092	0.04725	0.07803	0.05116	0.02860	0.04741 ± 0.01596	0.04057
B	(ng/ml)	85	40	75	42	75	108	78	72 ± 24	65
β	(min.$^{-1}$)	0.00428	0.00079	0.00247	0.00108	0.00718	0.00427	0.00343	0.00336 ± 0.00219	0.00275
c_{max}	(ng/ml)	251	414	316	409	228	368	261	321 ± 77	313
t_{max}	(min.)	10	6	6	6	10	6	6	7 ± 2	6
$(t_{\frac{1}{2}})_a$	(min.)	1.7	0.4	1.0	0.3	4.5	1.1	0.7	1.4 ± 1.5	1.0
$(t_{\frac{1}{2}})_\alpha$	(min.)	20.4	16.5	13.6	14.7	8.9	13.5	24.2	16.0 ± 5.0	17.1
$(t_{\frac{1}{2}})_\beta$	(min.)	161.9	877.2	280.6	641.7	96.5	162.3	202.0	346.0 ± 295.7	252.0
k_{12}	(min.$^{-1}$)	0.01410	0.03075	0.03095	0.03325	0.03082	0.02587	0.01221	0.02542 ± 0.00868	0.02196
k_{21}	(min.$^{-1}$)	0.01210	0.00430	0.01153	0.00513	0.01381	0.01493	0.01018	0.01028 ± 0.00411	0.00910
k_{13}	(min.$^{-1}$)	0.01199	0.00772	0.01091	0.00995	0.04058	0.01463	0.00964	0.01506 ± 0.01146	0.01226
V_c	(l)	50.4	34.5	40.4	33.9	20.2	34.1	55.7	38.5 ± 11.8	41.9
V_c	(l/kg)	0.61	0.51	0.57	0.49	0.25	0.42	0.68	0.50 ± 0.14	
$(V_d)_\beta$	(l)	141.2	337.3	178.6	312.0	114.3	117.0	156.7	193.9 ± 92.3	186.8
$(V_d)_\beta$	(l/kg)	1.7	5.0	2.5	4.5	1.4	1.4	1.9	2.6 ± 1.5	
Cl_{tot}	(ml/min.)	604.3	266.4	441.3	336.9	820.7	499.6	537.3	500.9 ± 182.5	513.8
r (correlation coefficient)		0.995	0.998	0.993	0.986	0.998	0.993	0.990		1.000

curve after a dose of 25.0 mg is not 200% (= theoretical value) of that obtained after a dose of 12.5 mg but only approximately 165%. The difference between the mean values of the areas under the curve proved to be significant ($p < 0.05$).
An alternative explanation could be the following. If the assumption is correct that enterohepatic cycling occurs to a considerable extent for thiazinamium cations, it seems to be possible that this process is inhibited by high amounts of the drug, because anticholinergic drugs may interfere on several stages of this process (*e.g.* bile production, motility of the small intestines). Such an inhibition would lead to a relatively smaller area under the curve after giving a higher dose.
After a dose of 25.0 mg in most of the patients the plasma concentration at t = 420 min. is relatively higher than after the 12.5 mg dose, which would support this hypothesis.

Determination of pharmacokinetic parameters using the NONLIN programme

The pharmacokinetic constants have also been calculated by means of the NONLIN programme using the equation XV (see Section 3.3.1.). The same weighting factor was applied. The results of these calculations are given in Table 5 for the 12.5 mg dose and in Table 8 for the 25.0 mg dose. Generally speaking, the conclusions drawn for the 6.25 mg dose in Section 3.3.1 are also valid here.

3.3.3.2. Determination of the bioavailability after oral administration

Determination of pharmacokinetic parameters using the NAFFIT-1 programme

The pharmacokinetic parameters obtained in six of the patients in this experiment by employment of the NAFFIT-1 programme are given in Table 9 (for equations and model see Section 3.3.1). The data for the other four patients (A.O., K.W., A.V. and E.D.) have already been given in Table 6. Examples of plasma concentration-time curves are given in Fig. 7 and Fig. 9.
Generally speaking the results of this experiment confirm the conclusions given in Section 3.3.3.1. However, attention should be drawn to the fact that particularly in this group a rather large variation was found in the profiles of the plasma concentration-time curves and hence in the pharmacokinetic parameters. It seems to be more pronounced with a dose of 25.0 mg as compared to a dose of 6.25 mg and 12.5 mg.

TABLE 8: *Pharmacokinetic parameters of thiazinamium methylsulphate after intramuscular injection of a dose of 25.0 mg in seven patients. NONLIN programme. Weighting factor* $W = {}^1/\sqrt{y_i}$.

Patient		A.O.	K.W.	A.V.	E.D.	N.S.	E.W.	E.P.	mean ± S.D.	averaged plasma conc. curve
c_p^o	(ng/ml)	525	996	352	450	362	899	516	586 ± 258	527
t_o	(min.)	1.8	0.0	2.4	2.5	2.8	2.7	0.2	1.8 ± 1.2	1.5
k_a	(min.$^{-1}$)	1.0733	0.47264	2.1264	0.73368	3.1733	2.8018	0.36608	1.5353 ± 1.1539	0.76865
A	(ng/ml)	368	865	310	407	336	818	357	494 ± 239	439
α	(min.$^{-1}$)	0.04700	0.04299	0.01552	0.01881	0.02551	0.04194	0.03736	0.03273 ± 0.01263	0.02823
B	(ng/ml)	157	131	42	43	26	81	159	91 ± 57	88
β	(min.$^{-1}$)	0.00625	0.00383	0.00018	0.00048	0.00005	0.00268	0.00315	0.00237 ± 0.00230	0.00268
c_{max}	(ng/ml)	450	680	334	395	335	792	388	482 ± 181	457
t_{max}	(min.)	6	10	6	10	6	6	10	8 ± 2	6
$(t_{\frac{1}{2}})_a$	(min.)	0.6	1.5	0.3	0.9	0.2	0.2	1.9	0.8 ± 0.7	0.9
$(t_{\frac{1}{2}})_\alpha$	(min.)	14.7	16.1	44.7	36.8	27.2	16.5	18.5	24.9 ± 11.8	24.5
$(t_{\frac{1}{2}})_\beta$	(min.)	110.9	180.9	3850.0	1443.8	13860.0	258.6	220.0	442.8 ± 562.2[1]	258.6
k_{12}	(min.$^{-1}$)	0.01888	0.01951	0.01230	0.01301	0.02300	0.02032	0.01822	0.01789 ± 0.00389	0.01307
k_{21}	(min.$^{-1}$)	0.01844	0.00898	0.00201	0.00254	0.00188	0.00622	0.01369	0.00768 ± 0.00643	0.00695
k_{13}	(min.$^{-1}$)	0.01593	0.01833	0.00139	0.00405	0.00068	0.01808	0.00860	0.00958 ± 0.00782	0.01089
V_c	(l)	61.8	32.6	92.2	72.1	89.6	36.1	62.9	63.9 ± 23.4	61.6
V_c	(l/kg)	0.75	0.48	1.30	1.04	1.12	0.45	0.77	0.84 ± 0.32	
$(V_d)_\beta$	(l)	157.5	155.9	711.5	607.7	1216.9	243.4	171.5	466.2 ± 402.2	250.2
$(V_d)_\beta$	(l/kg)	1.9	2.3	10.0	8.8	15.2	3.0	2.1	6.2 ± 5.2	
Cl_{tot}	(ml/min.)	984.5	597.6	128.2	292.0	60.9	652.7	540.9	465.3 ± 325.3	670.8
r (correlation coefficient)		0.994	0.998	0.972	0.995	0.985	0.996	0.989		0.999

[1] = without patients A.V. and N.S.

TABLE 9: *Pharmacokinetic parameters of thiazinamium methylsulphate after intramuscular injection of a dose of 25.0 mg in ten patients. NAFFIT-1 programme (N.B. Four of them, namely A.O., K.W., A.V. and E.D. are already listed in Table 6).*

Patient	F.K.	J.A.M.	D.M.	D.H.	O.V.	S.W.	mean ± S.D. (n = 6)	mean ± S.D. (n = 10)	averaged plasma conc. curve (n = 10)
Age (years)	33	60	56	52	18	22	40 ± 18	41 ± 18	
Body weight (kg)	78	88	71	63	83	66	75 ± 10	74 ± 8	
Height (m)	1.78	1.83	1.68	1.73	1.82	1.83	1.78 ± 0.06	1.77 ± 0.08	
Dose* (mg)	32.44	32.44	32.44	32.44	32.44	32.44	32.44	32.44	32.44
Dose* (mg/kg)	0.4159	0.3686	0.4569	0.5149	0.3908	0.4915	0.4398 ± 0.0577	0.4438 ± 0.0484	
c_p^o (ng/ml)	406	920	536	353	1200	311	621 ± 359	599 ± 299	515
k_a ($min.^{-1}$)	0.09770	0.14815	0.28791	0.75204		0.10940	0.27904 ± 0.27505[1]	0.36578 ± 0.22518[1]	0.27211
A (ng/ml)	313	751	438	326	1065	253	524 ± 319	513 ± 266	397
α ($min.^{-1}$)	0.03780	0.07185	0.03708	0.01664	0.14463	0.01317	0.05353 ± 0.04928	0.04245 ± 0.003976	0.04016
B (ng/ml)	93	173	98	27	135	58	97 ± 52	86 ± 44	118
β ($min.^{-1}$)	0.00200	0.00803	0.00412	0.00203	0.01335	0.00126	0.00513 ± 0.00472	0.00372 ± 0.00401	0.00486
c_{max} (ng/ml)	179	319	367	318	572	216	329 ± 139	381 ± 158	344
t_{max} (min.)	20	12	8	6	6	20	12 ± 6	10 ± 5	10
$(t_{\frac{1}{2}})_a$ (min.)	7.1	4.7	2.4	0.9		6.3	4.3 ± 2.6[1]	3.0 ± 2.4[1]	2.5
$(t_{\frac{1}{2}})_\alpha$ (min.)	18.3	9.6	18.7	41.6	4.8	52.6	24.3 ± 18.8	26.4 ± 15.4	17.3
$(t_{\frac{1}{2}})_\beta$ (min.)	346.5	86.3	168.2	341.4	51.9	550.0	257.4 ± 189.8	449.9 ± 498.8	142.6
k_{12} ($min.^{-1}$)	0.02219	0.03116	0.01600	0.00479	0.06120	0.00618	0.02359 ± 0.02092	0.01979 ± 0.01647	0.01700
k_{21} ($min.^{-1}$)	0.01020	0.02007	0.01015	0.00315	0.02812	0.00348	0.01253 ± 0.00981	0.00935 ± 0.00847	0.01295
k_{13} ($min.^{-1}$)	0.00741	0.02875	0.01506	0.01073	0.06867	0.00477	0.02257 ± 0.02411	0.01704 ± 0.01957	0.01507
V_c (l)	79.9	35.3	60.5	91.9	27.0	104.3	66.5 ± 31.1	65.3 ± 26.2	63.8
V_c (l/kg)	1.02	0.40	0.85	1.46	0.33	1.58	0.94 ± 0.52	0.92 ± 0.43	
$(V_d)_\beta$ (l)	296.1	126.3	221.2	485.8	139.0	394.6	277.2 ± 143.3	323.0 ± 162.3	195.4
$(V_d)_\beta$ (l/kg)	3.80	1.43	3,12	7.71	1.67	5.98	4.0 ± 2.5	4.6 ± 2.6	
Cl_{tot} (ml/min.)	592.1	1014.9	911.1	986.1	1854.1	497.5	976.0 ± 480.3	780.2 ± 449.7	962.2
r (correlation coefficient)	0.976	0.911	0.992	0.977	0.985	0.866			0.990

[1] = without patient O.V.

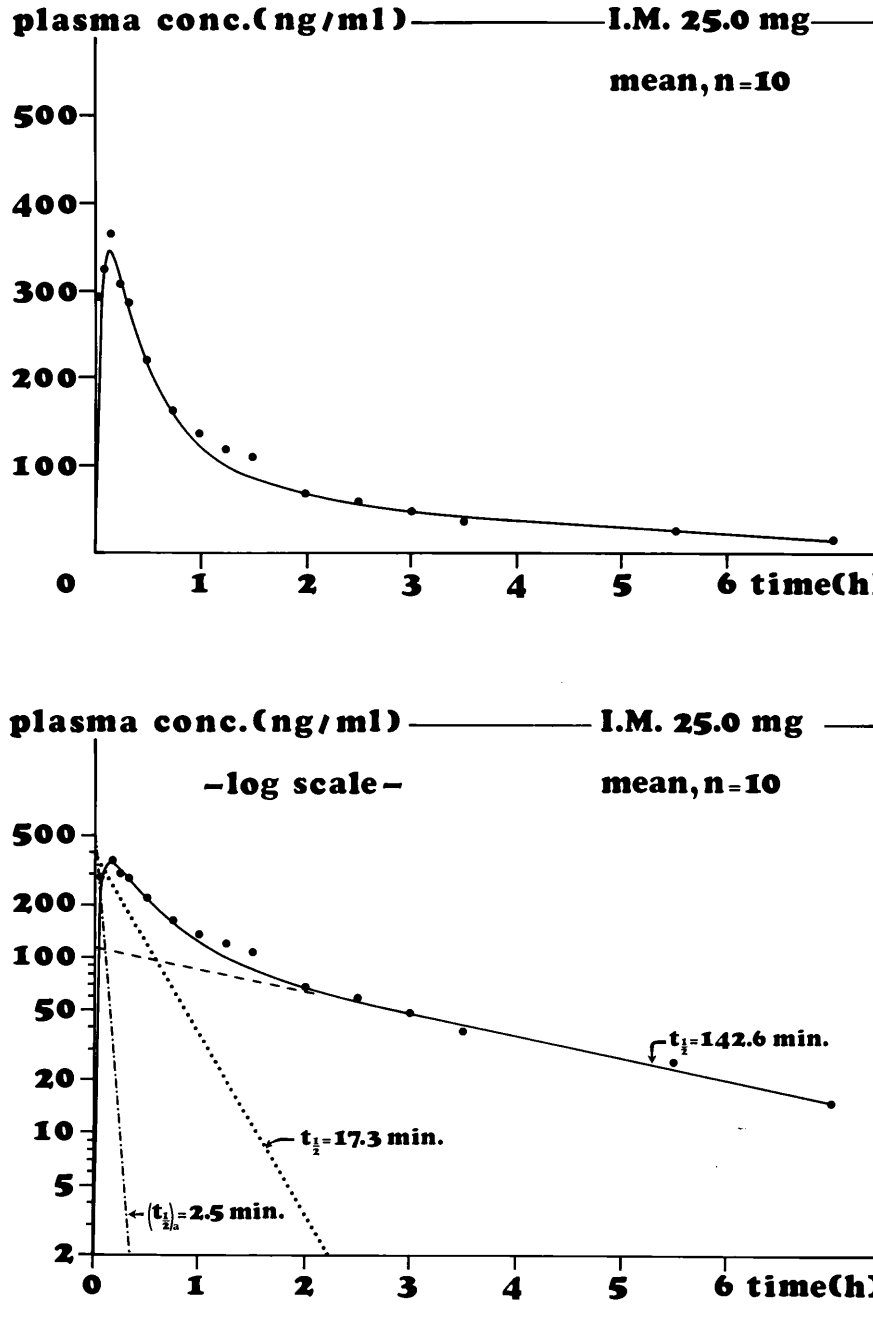


FIG. 9: *Plasma concentration-time curve after intramuscular injection of a dose of 25.0 mg. NAFFIT-1 programme. Averaged data curve (n = 10). Above on linear scale, below on semi-logarithmic scale.*

The curves obtained with the dose of 25.0 mg can in fact be divided into three types.
In the curves of the first type the maximum plasma concentration was found in the 3 (or 6) min. sample, after which there was a steep fall. Absorption from the muscle seems to be extremely fast so that the curves tend to attain the same profile as those obtained after intravenous injection (see Section 2.3.). Typical examples are found in the curves of patient O.V., and less pronounced in K.W. and D.H. In addition the pharmacokinetic parameters of the drug in patient O.V. are almost identical to those found in the experiment with intravenous administration. As the intramuscular position of the injection needle was always verified, it may be concluded that in these patients injection took place in a large capillary bed with high capillary blood flow.
The curves of the second type have the same profiles as those described in Section 3.3.1 and Section 3.3.2. This time the rate of absorption is such that the peak in the plasma concentration-time curve appears between 8 and 12 min. after injection (absorption half-life between 1.5 and 3 min.). This is the most frequently encountered type of curve after intramuscular injection of thiazinamium methylsulphate.
In the curves of the third type, the maximum plasma concentration was found not earlier than after appr. 20 min. (*e.g.* F.K. and S.W.). The rate of absorption is now much lower than in the previously described instances ($k_a < 0.10000$ and hence an absorption half-life of more than 6 min.). As a consequence the maximum plasma concentration was appr. 200 ng/ml, which is much lower than in the other patients. An explanation for this could be that in patients F.K. and S.W. the drug was injected in tissue with a relatively low amount of capillaries and with low capillary blood flow (*e.g.* fat tissue).
These findings suggest, that injection technique, and particularly the exact injection site affect the final profile of the plasma concentration-time curve after intramuscular administration of thiazinamium methylsulphate, which is in agreement with findings on other drugs by REEVES and others (1974) and the general terms stated by EVANS and others (1973) (see Introduction).

Determination of pharmacokinetic parameters using the NONLIN programme

The pharmacokinetic parameters obtained in six of the patients by employment of the NONLIN programme are given in Table 10 (for equation and model see Section 3.3.1.). The data for patient A.O., K.W., A.V. and E.D. have already been given in Table 8. Comparison of Table 9 with Table 10 demonstrates that in general there exists a satisfactory correlation between the outcomes obtained by the NAFFIT-1 and NON-

TABLE 10: *Pharmacokinetic parameters of thiazinamium methylsulphate after intramuscular injection of a dose of 25.0 mg in ten patients. NONLIN-programme. Weighting factor* $W = 1/\sqrt{y_i}$ *(N.B. Four of the patients, namely A.O., K.W., A.V. and E.D. are already listed in Table 8).*

Patient		F.K.	J.A.M.	D.M.	D.H.	O.V.	S.W.	mean ± S.D.[1]	mean ± S.D.[2]	averaged plasma conc. curve (n = 10)
c_p^o	(ng/ml)	362	817	421	438	3000	258	882 ± 1054	762 ± 818	464
t_o	(min.)	0.4	0.9	0.6	0.1	0.1	0.3	0.4 ± 0.3	0.9 ± 1.0	0.1
k_a	(min.$^{-1}$)	0.10483	0.19252	0.49242	3.9631	3.0218	0.15628	1.3218 ± 1.7128	1.2337 ± 1.3486	0.37507
A	(ng/ml)	257	642	385	178	2742	211	736 ± 997	637 ± 768	310
α	(min.$^{-1}$)	0.04107	0.06970	0.02131	0.06095	0.32842	0.01027	0.08862 ± 0.11963	0.06560 ± 0.09445	0.03880
B	(ng/ml)	105	175	36	260	258	47	147 ± 100	125 ± 87	154
β	(min.$^{-1}$)	0.00349	0.00775	0.00125	0.01218	0.02188	0.00098	0.00792 ± 0.00807	0.00583 ± 0.00680	0.00607
c_{max}	(ng/ml)	166	362	350	401	1288	202	462 ± 416	463 ± 322	345
t_{max}	(min.)	20	10	6	3	3	15	10 ± 7	9 ± 5	6
$(t_{\frac{1}{2}})_a$	(min.)	6.6	3.6	1.4	0.2	0,2	4.4	2.7 ± 2.6	2.0 ± 2.1	1.8
$(t_{\frac{1}{2}})_\alpha$	(min.)	16.9	9.9	32.5	11.4	2.1	67.5	23.4 ± 23.9	25.3 ± 19.9	17.9
$(t_{\frac{1}{2}})_\beta$	(min.)	198.6	89.4	554.4	56.9	31.7	707.1	273.0 ± 287.0	722.4 ± 1182.9	114.2
k_{12}	(min.$^{-1}$)	0.02021	0.03073	0.01061	0.01395	0.15311	0.00481	0.03890 ± 0.05665	0.02971 ± 0.04391	0.01403
k_{21}	(min.$^{-1}$)	0.01439	0.02102	0.00297	0.04113	0.04824	0.00267	0.02174 ± 0.01923	0.01624 ± 0.01659	0.01693
k_{13}	(min.$^{-1}$)	0.00996	0.02570	0.00898	0.01805	0.14895	0.00377	0.03590 ± 0.05592	0.02551 ± 0.04405	0.01391
V_c	(l)	89.6	39.7	77.1	74.1	10.8	125.7	69.5 ± 39.9	67.6 ± 33.1	69.9
V_c	(l/kg)	1.15	0.45	1.09	1.18	0.13	1.91	0.99 ± 0.62	0.95 ± 0.51	
$(V_d)_\beta$	(l)	255.8	131.7	553.7	109.8	73.6	483.2	268.0 ± 204.7	324.0 ± 239.2	160.2
$(V_d)_\beta$	(l/kg)	3.3	1.5	7.8	1.7	0.9	7.3	3.8 ± 3.1	4.6 ± 3.5	
Cl_{tot}	(ml/min.)	892.4	1020.3	692.4	1337.5	1608.7	473.9	1004.2 ± 416.6	802.8 ± 459.7	972.3
r (correlation coefficient)		0.975	0.953	0.993	0.990	0.997	0.892			0.997

[1] = mean value of the six patients listed in this table
[2] = mean value of all ten patients.

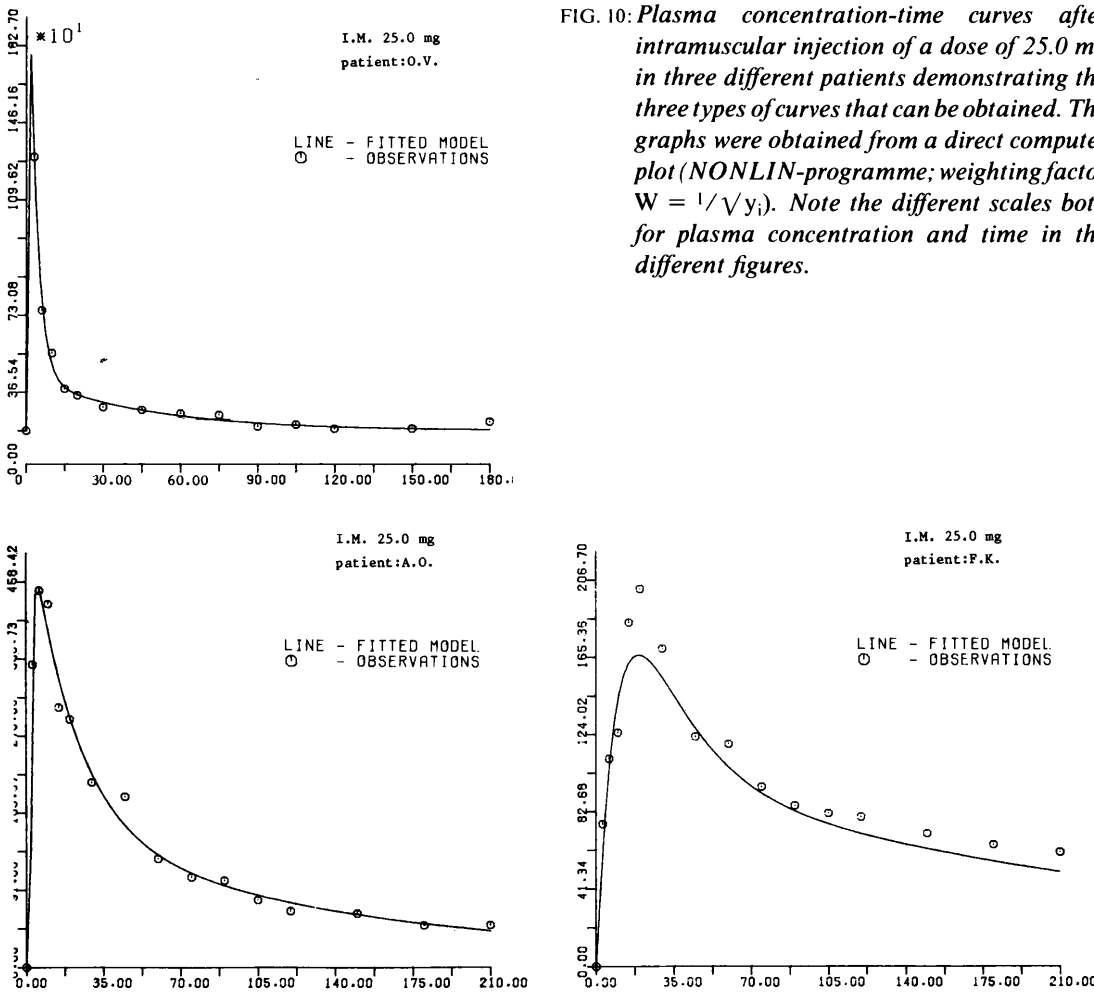


FIG. 10: *Plasma concentration-time curves after intramuscular injection of a dose of 25.0 mg in three different patients demonstrating the three types of curves that can be obtained. The graphs were obtained from a direct computer plot (NONLIN-programme; weighting factor* $W = 1/\sqrt{y_i}$). *Note the different scales both for plasma concentration and time in the different figures.*

LIN programme. The points made in Section 2.3. are also valid in this case. Examples of plasma concentration-time curves of the three types mentioned above are given in Fig. 10.

3.3.3.3. The influence of muscle contraction on the bioavailability

During the early stages of our investigations we often observed a second peak in the plasma concentration-time curve obtained after intramuscular administration of 25.0 mg in a series of seven patients. This second peak was reached between 20 and 30 min. after injection. In most patients it was lower than the first one, but in some it was higher. Although no explanation seemed available at first instance, a critical analysis of all circumstances of

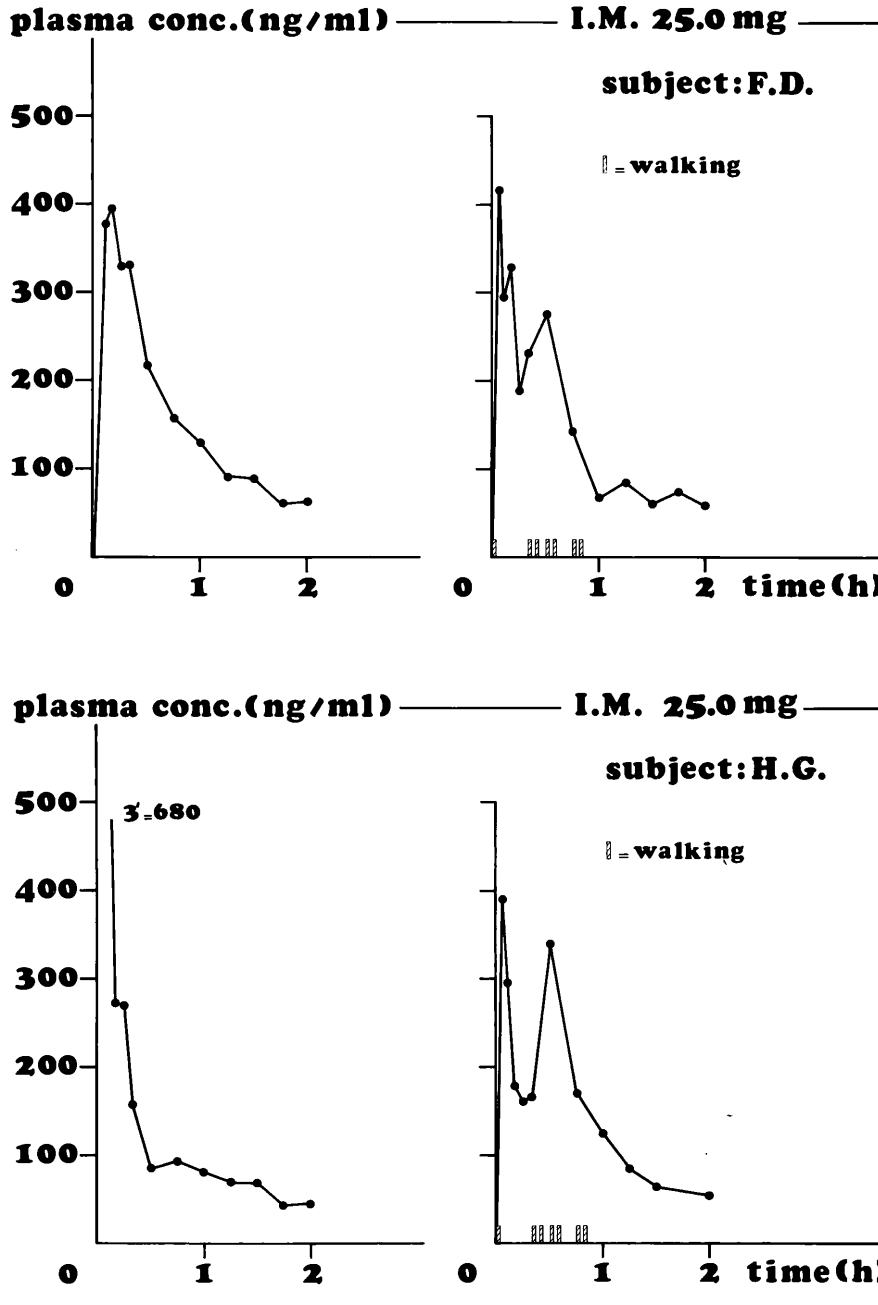


FIG. 11: *Two typical examples of plasma concentration-time curves obtained after an intramuscular injection of a dose of 25.0 mg. Left: Subject in resting position. Right: Resting position interrupted by small periods of walking as indicated by the bars.*

the experiment brought out that the second maximum coincided with a short period of walking to another room in the hospital, which was done for practical reasons. We then decided to examine this phenomenon in more detail in seven volunteers by performing an experiment as described under 3.2.3.3. Two examples of plasma concentration-time curves obtained in this experiment are given in Fig. 11. From the curves it can be concluded that walking (*i.e.* increased muscle activity) during the first two minutes after injection does not influence the profile of the curve. However, the second period of walking (20 to 22 min. after injection) was immediately followed by a sharp increase of the plasma concentration. As was pointed out in the previous chapters, this period falls in the distribution phase (α-phase). The other periods of walking do not measurably influence the shape of the curve.

Obviously the moment t = 20 min. is rather critical, with the distribution process being at its full height. At this stage an increase in muscle capillary blood flow apparently results in a marked increase of the absorption of the drug from the drug depot at the site of injection, which caused the second peak. These facts lead us to think that the minor increases of plasma concentrations observed in some of the other curves of the experiments we described in the previous chapters were perhaps caused by minor increase of muscle activity of the patients. As the semi-logarithmic curve (see *e.g.* Fig. 9) shows, the absorption process is almost complete at the later periods of walking and hence increase of muscle capillary blood flow there does not result in any notable increase of the plasma concentration of thiazinamium methylsulphate.

Results found by other authors indicate that massage and body movement can lead to increased absorption from the muscle (BALLARD, 1968).

SUNDWALL (1960) reported that absorption of the quaternary ammonium compound N-methylpyridinium-2-aldoxime methylsulphate varies greatly from subject tot subject which was originally ascribed to different dephts of injection. Considerable fluctuations in plasma concentrations were observed, too. Later on, the author (SUNDWALL, 1974, *Personal Communication*) agreed that these fluctuations may have been caused by the fact that free movement (including walking) was allowed during the experiment.

Conclusion

For a low dose (6.25 mg) the absolute bioavailability of an intramuscular injection was found to be 100%. By comparison of the areas under the plasma concentration-time curves obtained after giving 12.5 mg and 25.0 mg to the same patients we found that bioavailability during the first seven hours after administration was relatively lower after giving the highest dose. Two explanations are suggested.

The plasma concentration-time curves are resolved into two exponential components, suggesting an open two-compartment model with absorption phase.

Absorption was found to be extremely fast. Peak concentrations were always reached within 20 min., but in the majority of cases they were found to be between 8 and 10 min. In a few instances the first sample (t = 3 min.) proved to contain the highest concentration, which resulted in a curve with a profile like that found after intravenous injection. These findings suggest that solubility of the drug in interstitial fluid is of prime importance for rapid absorption. Neither lipid insolubility nor the small amount of intercellular pores in the membrane – if this hypothesis is correct – are rate-limiting factors.

Injection technique and the injection site seem to be important in relation to the final profile of the plasma concentration curve.

Distribution was found to be very fast as well, with a $(t_{\frac{1}{2}})_{\alpha}$ of appr. 20 min., and apparent volumes of distribution for the central compartment were small: V_c is appr. 40 to 60 l.

Muscle activity, and hence increased capillary blood flow, during the α-phase may result in a second peak in the plasma concentration-time curve.

The distribution phase is followed by an elimination phase with a much longer half-life, namely appr. 300 to 600 min. and a $(V_d)_{\beta}$ of appr. 200 to 400 l.

In all experiments (6.25, 12.5 and 25.0 mg) plasma concentrations were zero or approaching zero at the end of the experiment at t = 420 min.

References

BALLARD, B. E. (1968), *J. Pharm. Sci. 57,* 357

BALLARD, B. E. and E. NELSON (1965) in: *"Remington's Pharmaceutical Sciences"*,, 13th ed., p. 627, Mack Publ. Co., Easton, Pa.

BORK, L. E. VAN, J. H. G. JONKMAN, R. A. DE ZEEUW, N. G. M. ORIE, R. PESET and K. DE VRIES (1977a), *Ned. T. Geneesk.*, in press

BORK, L. E. VAN, J. H. G. JONKMAN, R. A. DE ZEEUW, N. G. M. ORIE, R. PESET and K. DE VRIES (1977b), *Scand. J. Resp. Dis.*, submitted for publication

DUNDEE, J. W., J. A. S. GAMBLE and R. A. E. ASSAF (1974), *Lancet, ii,* 1461

EDITORIAL (1975), *Lancet, i,* 261

EVANS, E. F., J. D. PRACTOR, M. J. FRATKIN, J. VELANDIA and A. J. WASSERMAN (1973), *Clin. Pharmacol. Ther. 17,* 44

FOLDES, F. F., I. M. BROWN and J. N. LUNN (1962), *Anesthesiology 23,* 213

GREENBLATT, D. J. and J. KOCH-WESER (1976), *N. Engl. J. Med. 295,* 542

GREENBLATT, D. J., R. I. SHADER and J. KOCH-WESER (1974), *N. Engl. J. Med. 291,* 1116

HILLESTAD, L., T. HANSEN, H. MELSOM and A. DRIVENES (1974), *Clin. Pharmacol. Ther. 16,* 479

HOGBEN, C. A. M. (1971) in: *"Concepts in Biochemical Pharmacology"*, Part I, p. 1 (B. B. Brodie and J. R. Gilette, Eds.), Springer Verlag, Berlin, New York
JONKMAN, J. H. G., L. E. VAN BORK, R. A. DE ZEEUW and N. G. M. ORIE (1976), *Lancet i,* 693
KERCKHOFFS, H. P. M. and T. HUIZINGA (1967), *Pharm. Weekbl. 102,* 1183
KARLSSON, E., P. COLLSTE and M. D. RAWLINS (1974), *Europ. J. Clin. Pharmacol. 7,* 455
KOSTENBAUDER, H. B., R. P. RAPP, J. M. MCGOVERN, T. S. FOSTER, D. G. PERRIER, H. M. BLACKER, W. C. HULON and A. W. KINKEL (1975), *Clin. Pharmacol. Ther. 18,* 449
MÖLLER, J. and A. ROSÉN (1968), *Acta Med. Scand. 184,* 201
REEVES, D. S., M. J. BYWATER, R. WISE and V. B. WHITMARSH (1974), *Lancet, ii,* 1421
SCHOU, J. (1971) in: *"Concepts in Biochemical Pharmacology"*, Part I, p. 46, (B. B. Brodie and J. R. Gilette, Eds.), Springer Verlag, Berlin, New York
SIDELL, F. R. and W. A. GROFF (1971), *J. Pharm. Sci. 60,* 1224
SUND, R. B. and J. SCHOU (1974), *Acta Pharmacol. Toxicol. 21,* 313
SUNDWALL, A. (1960), *Biochem. Pharmacol. 5,* 225
SUNDWALL, A., J. VESSMAN and B. STRINDBERG (1973), *Europ. J. Clin. Pharmacol. 6,* 191
SUNDWALL, A. (1974), *Personal Communication*
WILDER, B. J., E. E. SERRANO, E. RAMSEY, R. A. BUCHANAN (1974), *Clin. Pharmacol. Ther. 16,* 507

4

Bioavailability after oral administration in man

Contents

4.1. Introduction

As regards their gastrointestinal absorption, quaternary ammonium compounds take up a special position among drugs due to their physico-chemical properties. As a consequence of the presence of the quaternary ammonium group in the molecule these compounds have high pK_a values and are ionized at any pH value. Hence, the liposolubility at physiological pH in the stomach and in the small intestines is almost zero (see also Part I, Section 2.8 "Distribution coefficient" and Table 2 in that section). This is in sharp contrast to most drug molecules, which are weak electrolytes and are generally well absorbed in the unionized (uncharged) form. BRODIE, HOGBEN, SCHANKER and their coworkers proposed the pH-partition hypothesis to explain the absorption of the unionized drugs. Their theory states that since the gastrointestinal epithelium is lipoidal in nature, only lipophilic substances are able to pass the intestinal membrane by *passive diffusion* and enter the blood stream (see *e.g.* HOGBEN, 1957; BRODIE, 1964; SCHANKER, 1962, 1971). This means that after oral application absorption of many drug molecules is pH dependent. HOGBEN and others (1959) suggested that the ratio of unionized to ionized forms should not be less than 1 to 200 for reaching sufficient gastrointestinal absorption, and

that the rate and efficiency of absorption depends solely upon the amount of unionized molecules.

Because of their ionized state at all physiological pH values, absorption of quaternary ammonium compounds by passive diffusion as postulated in the pH-partition hypothesis is assumed to be negligible. Based on *"in situ"* experiments, obtained by using isolated loops of rat intestines, LEVINE and coworkers found results suggesting that quaternary ammonium compounds *can* be absorbed – albeit to a small extent – after oral administration in spite of the presence of the strongly polar group (LEVINE, 1961, 1966; LEVINE and PELIKAN, 1961, 1964; LEVINE and others, 1955; KUNZE and others, 1971).

On the other hand these investigators (LEVINE and others, 1955) suggested that the limited absorption of the quaternary ammonium compound benzomethamine was due to formation of a nonabsorbable complex with mucin present in the intestines. CAVALLITO and O'DELL (1958) studied and reported comprehensively about modification – from physical-chemical and physiological bases – of rates of gastrointestinal absorption of quaternary ammonium compounds in animals and *"in vitro"*. In an *"in vitro"* experiment these authors found that mucin has a marked retarding influence on the dialysis of a quaternary ammonium compound (IN 391). They also suggested that a quaternary ammonium compound (IN 414) that is biologically relatively inert could increase the oral effectiveness of a pharmacologically effective one, probably by blocking sites of loss of the latter in the gastrointestinal tract. LEVINE and PELIKAN (1961) reported increased absorption when a period of fasting had preceded.

It is only in a limited number of papers that absorption of quaternary ammonium compounds in *man* is described.

Several methods have been used to get an indication of the absorption. HERXHEIMER and HAEFELI (1966) used the method of monitoring pharmacological responses after oral administration of hyoscine butylbromide (= butylscopolamine bromide) and concluded that doses up to 600 mg are inactive in man.

Some authors adopted the method of measurement of the urinary excretion of a quaternary ammonium ion in order to get some impression of the amount of drug absorbed. On anisotropine methylbromide PFEFFER and associates (1968b) found a cumulative urinary excretion of 3.5 to 11.4% of the dose, which points to incomplete absorption. For propantheline bromide PFEFFER and coworkers (1968a) found a cumulative urinary excretion of 1.6 to 5.7% of the dose. HARINGTON (1953) found that absorption after oral administration of hexamethonium (administered as bromide, chloride, bitartrate or methylsulphate) was poor, urinary excretion not exceeding 10% of the dose when the drug was given after breakfast. The absorption could be greatly increased (up to a factor of about 4) by giving the dose in the fasting state, but still remained incomplete.

Several of these studies are of limited value because they do not account for possible metabolites, biliary excretion and interindividual and intraindividual variation.

The group of BEERMANN, HELLSTRÖM and ROSÉN (1970 to 1972) employed a radiochemical technique in man to measure the degree of absorption of several quaternary ammonium ions: methylatropine, methylscopolamine, butylscopolamine, Acabel® (the benzilic acid ester of N, N-dimethyl-2-hydroxy-methylpiperidinium methylsulphate) and propantheline. For methylatropine, methylscopolamine and Acabel® an absorption of 15 to 25% was found, while very little butylscopolamine was taken up. Hydrolysis of propantheline in the upper small intestine prevented determination of the absorption of this drug.

Only some publications deal with the absorption of quaternary ammonium compounds in man measured by determining plasma concentrations of the drugs (SUNDWALL, 1961; KONDRITZER and others, 1968; SIDELL and GROFF, 1969, 1971; SIDELL and others, 1972; SUNDWALL and others, 1973; COPER and others, 1974). The latter method is much more reliable than *"in situ"* animal experiments because they take such factors into account as adsorption to intestinal mucus and "first pass effect".

There are a few hypotheses which try to explain why it *is* possible that quaternary ammonium ions can be absorbed, which would seem impossible on the basis of pH-partition (passive diffusion).

The first hypothesis assumes that quaternary ammonium ions, dissolved in aqueous solution, can pass through small water-filled "pores" in the lipophilic membrane and reach the blood stream. Such a "pore" is a continuous aqueous phase permeating the plasma membrane. The hypothetical radius of an intercellular "pore" is about 3.5 Å (see review of HOGBEN, 1971). This implies that the radius of these "pores" in the capillary membrane in the intestines is much smaller than the radius of those in the muscles (see also Section 3.3.1.). As a consequence of this *"filtration process"*, as it is called, hydrophilic substances (inorganic or organic electrolytes) with a molecular weight of up to approximately 200, depending on molecular structure, can be absorbed. This process is also called "convective absorption", *e.g.* by RITSCHEL, 1976, who gave a comprehensive survey of absorption processes in general.

A second hypothesis was given by LEVINE and SPENCER (1961). These authors postulated that quaternary ammonium compounds may be passing the lipophilic membrane after *forming a complex with a "phosphatido-peptide" fraction* present in the small intestines. They found that the absorption of benzomethamine (= N-diethyl-aminoethyl-N'-methylbenzilamide methylbromide) could be increased by concurrently administering this "phosphatido-peptide" fraction. This is a tissue fraction which can be isolated in a special way from small intestines but also from brain, heart, liver, lung, spleen, pancreas or kidney. However, this hypothesis has not yet

been confirmed for other quaternary ammonium compounds and seems to be of little practical importance.

More recently, a third hypothesis was formulated by several investigators, which rests on the assumption that quaternary ammonium compounds can be absorbed as *ion pairs*. In Part II it was already pointed out that in general quaternary ammonium compounds can easily form ion pairs with several anions. Ion pairs are uncharged and generally more lipophilic, and they can easily be dissolved in weakly polar organic solvents (*e.g.* chloroform, n-octanol). This ability to form a lipid-soluble derivative from a strongly charged compound has led to attempts to improve oral absorption of quaternary ammonium compounds by giving them together with potential counter ions, the idea behind it being that the ion pairs thus formed will diffuse through the lipophilic part of the intestinal membrane. After absorption the ion pair is expected to split into the seperate ions in the blood stream (see Fig. 1). IRWIN and others (1969) reported an increased absorption of isopropamide in the presence of trichloroacetate. Mydriasis tests in mice demonstrated that both the rate and the efficiency of oral absorption of isopropamide were increased when its iodide was administered in combination with 10-fold or 50-fold molar excesses of trichloroacetate in solution. This effect was more pronounced with rather low doses of the drug. GAGINELLA and others (1973a) found that isopropamide iodide as such disappeared to a degree of 40% during 1 hour after placing the drug in the tied-off loop of the duodenum of the rat. Although the investigators were unable to find any drug in the blood, a mydriatic effect was observed

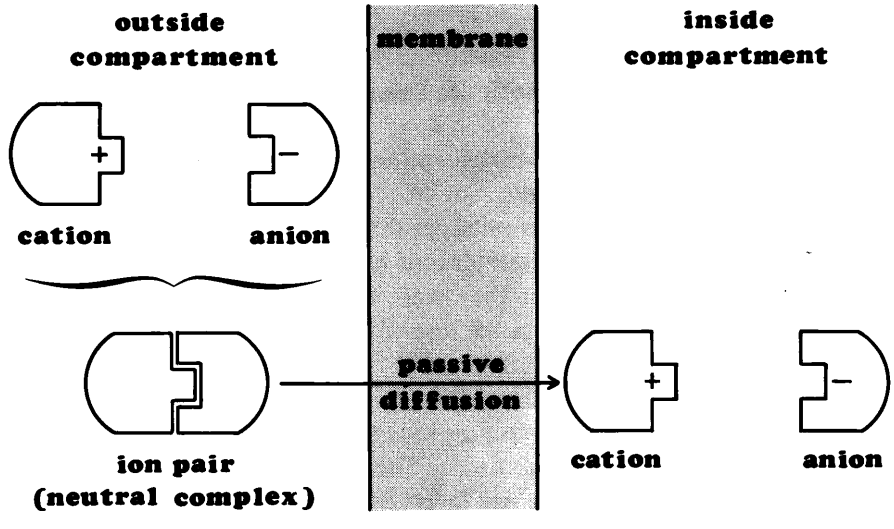


FIG. 1: *Schematic diagram characterizing ion pair absorption of a quaternary ammonium compound (= "cation"). (Redrawn from* RITSCHEL *(1976) with permission of the author.)*

after 15 min. Neither could any drug be found in the blood after an intravenous dose, but a mydriatic response was noted after 30 seconds (*N.B.* No detection limit was given for the method of analysis; although the authors did not mention it, it should be realized that isopropamide can be considered to be an ion pair in itself.). The same authors (1973b) also found that the gastrointestinal absorption of the isopropamide cation cannot be increased in the presence of bile salt molecules (glycocholate or taurodeoxycholate). GIBALDI and GRUNDHOFER (1973) found an increased absorption of N,N-bis(phenylcarbamoylmethyl)dimethylammonium chloride in the presence of an excess of both salicylate and trichloroacetate anions. A molar ratio of 1 : 90 was used in an *"in situ"* experiment. PLAKOGIANNIS and others (1970) with an *"in vitro"* intestinal method showed that sodium decylsulphate inhibited the transfer of certain pyridinium and quinolinium compounds, but on the other hand MASAKI and others (1973) found that sodium decylsulphate enhanced the absorption of 2-pyridinium aldoxime methyliodide. More recently TOMLINSON and DAVIS (1976) suggested ion pair formation as the reason of increased uptake of an anionic drug (sodium cromoglycate) by mucous membrane in the presence of quaternary ammonium ions. Their results were obtained by the human buccal absorption test. But it is still not clear in how far disappearance from solutions which are washed through the mouth gives a realistic impression of the possible extent of absorption, because adsorption to the mucus in the mouth is not taken into account in this test.

The group of FIESE, PERRIN, VALLNER and MROSZCZAK (1969, 1970) investigated the absorption of some drugs - not quaternary ammonium compounds - which are also ionized under physiological conditions *e.g.* dextramethorphan and tetracycline. They concluded that the increased absorption found after concurrent administration of trichloroacetate, was not due to ion pair formation with this counter ion but rather caused by an increased surface activity.

Although the concept of ion pair absorption sounds quite reasonable, a few critical remarks should be made.

Firstly, none of the above mentioned results of increased absorption in the presence of ion pair forming counter ions has been based on enhancement of plasma concentrations, but they were merely based on an increased disappearance of the drug from the intestinal fluid, which in itself is not a guarantee for better absorption.

Secondly, the amounts of counter ions required are generally very high. These high concentrations of counter ions must reduce dissociation of the ion pair in the gastrointestinal fluids. The counter ions are generally of exogenous nature and hence toxic levels may be reached.

This suggests that under physiological circumstances ion pair formation is not the major factor to explain absorption of quaternary ammonium ions in man. WAGNER (1971) said about ion pair absorption: "There is no proof

that any highly ionized drug is absorbed by such a mechanism. However, the idea is an attractive one and deserves investigation".

In conclusion it can be stated that for a variety of quaternary ammonium compounds absorption after oral administration has been reported to be incomplete, both in animal and man. ARIËNS and NELEMANS (1975) summarized the findings by saying that absorption from the gastrointestinal tract is "less complete and less regular than that of tertiary amines", and INNES and NICKERSON (1975) stated that "these drugs are poorly and unreliably absorbed after oral administration".
Several hypotheses exist to explain the absorption process of highly ionized drug compounds, but the mechanism is not understood as yet and practical methods to improve absorption have not yet been found.

This stimulated us to investigate the bioavailability of the quaternary ammonium compound thiazinamium methylsulphate after oral ingestion in man.
In the first and second part of this study we determined the relative bioavailability of two oral doses (300 and 900 mg respectively).
In the third part we investigated the intraindividual variation in the oral absorption of this drug.

4.2. Materials and methods

4.2.1. Investigations with a single dose of 300 mg

Subjects and conditions

The bioavailability of an oral dose of the drug (300 mg thiazinamium base, administered as methylsulphate) was compared to an intramuscular injection (25.0 mg) utilizing the plasma concentration-time curves in the same individual on two different days. The doses were given with at least two-day intervals which proved sufficient long to completely eliminate the previously given dose. Ten male patients, already described in Section 3.2.3.2, participated in this trial. The experiments were performed under the same conditions as described in that section and in Section 2.2. After oral administration the blood samples were taken at 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 330 and 420 min. intervals.

The dosage form

For *intramuscular injection* 1.0 ml of the solution as described in Section 2.2 was used.

For *oral administration* one coated tablet of Multergan forte® (SPECIA, Rhône Poulenc, Paris, France; all tablets from the same batch) was used. (*N.B.* This was the only commercially available dosage form for oral application in the Netherlands at the moment we started this trial.) Each tablet should theoretically contain 389.24 mg of thiazinamium methylsulphate, which equals 300 mg (= 0.9494 mmol) of thiazinamium base (= hydroxyde). The tablets (n = 20) were tested on content uniformity by determining the amount of drug in it. To this aim the method as described in Part I, Section 4.7.2 was used. It was found to be 97.9% ± 2.4 (S.D.)% of the stated amount.

The dissolution rate of the tablets was determined for seven tablets according to the U.S.P. XIX test (UNITED STATES PHARMACOPEIA, 1975).

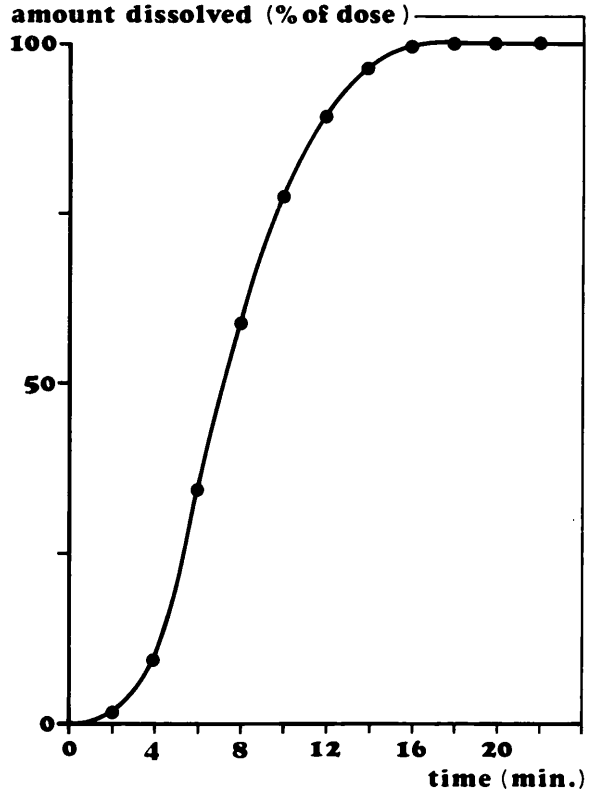


FIG. 2: *Typical example of a dissolution diagram of a coated tablet of Multergan forte®*

A typical example of a dissolution plot is given in Fig. 2. Within 16 ± 1 (S.D.) min. all of the drug was dissolved in 0.1 n hydrochloric acid at 100 rpm.
This dose will be referred to as the 300 mg dose

Determination of thiazinamium cations in plasma

Analysis was performed as described in Part II, Section 2.3. The concentration of the drug in plasma was expressed as nanogram of thiazinamium methylsulphate per ml plasma.

Determination of the relative bioavailability

The areas under the plasma concentration-time curves were determined by cutting and weighing of a standard type of high quality paper. The relative bioavailability *versus* an intramuscular injection of a dose of 25.0 mg was calculated as described in Chapter 1.

4.2.2. Investigations with a single dose of 900 mg

Patients and conditions of these experiments were exactly the same as those described in Section 4.2.1. Now the dose was administered as three of the coated tablets.
This dose will be referred to as the 900 mg dose.

4.2.3. Investigations with repeated doses of 900 mg

Subjects and conditions

To investigate the intraindividual variation in the bioavailability of the drug a dose of 900 mg was given to the same individuals on seven different days. Again, doses were given with at least two-day intervals. Eight male volunteers, aged between 23 and 36, mean 29 ± 5 (S.D.) years, weighing from 65 to 94 kg, mean 78 ± 10 (S.D.) kg participated in this study. Subjects were not on any medication during the experiment and were not suffering from any known illness. After 90 min. a light meal was eaten. Thereafter food and liquid consumption were *ad libitum* but standardized for each individual. The amount of drug which had been available in the body was

measured by determining the excretion of the parent drug in urine, collected during 24 hours. Excretion was shown to be completed within this period.

The dosage form

The same dosage form as described in Section 4.2.2 was used.

Determination of thiazinamium cations in urine

Analysis was performed as described in Part II, Section 2.4.

Determination of the relative bioavailability

The amount of the systemic bioavailability was estimated by multiplying the quantity of thiazinamium found in the urine, by a factor of 4.4 (from earlier excretion studies it had become clear that 22.6% ± 6.2 (S.D.)% of the drug in the body was excreted unchanged in urine; see Chapter 10).

4.3. Results and discussion

4.3.1. Investigations with a single dose of 300 mg

The plasma concentrations were found in general to be very low. Typical examples of plasma concentration-time curves are given in Fig. 3a and Fig. 3b. The curve for the averaged plasma concentrations for 10 patients is represented by Fig. 4a and Fig. 4b. The general remarks in Chapter 2, on the usefulness of an averaged plasma concentration-time curve are, of course, also valid here. Such a curve is generally smooth and does not reflect irregularities in absorption; moreover interindividual differences in the profiles of the curves may be lost.
The profiles of the individual curves indicate that in most cases the absorption started rather rapidly.
Soon the curves reached a plateau level, which was maintained during several hours. In some patients the curve was still at plateau level at the end of the experiment. This indicates that the absorption process was con-

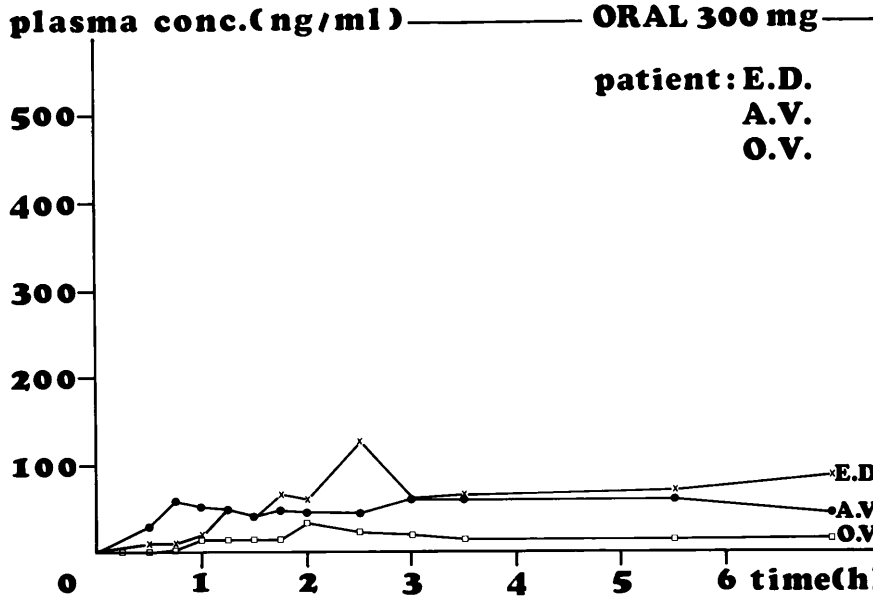


FIG. 3a: *Typical examples of individual plasma concentration-time curves obtained after oral administration of a dose of 300 mg. Linear scale.*

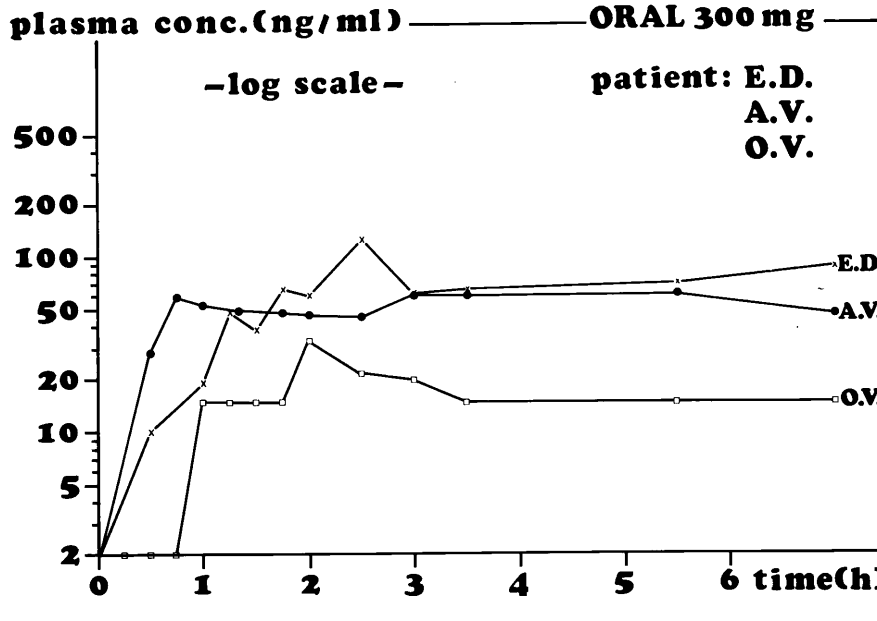


FIG. 3b: *The same data as in Fig. 3a, but drawn on semi-logarithmic scale.*

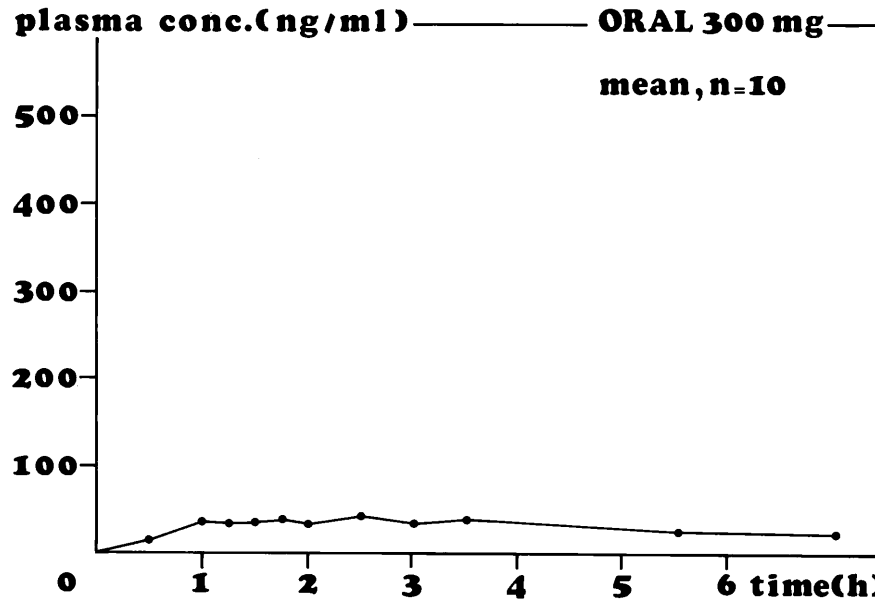


FIG. 4a : *Plasma concentrations (ng/ ml) after oral administration of a dose of 300 mg. The curve has been constructed from the averaged plasma concentrations of ten patients.*

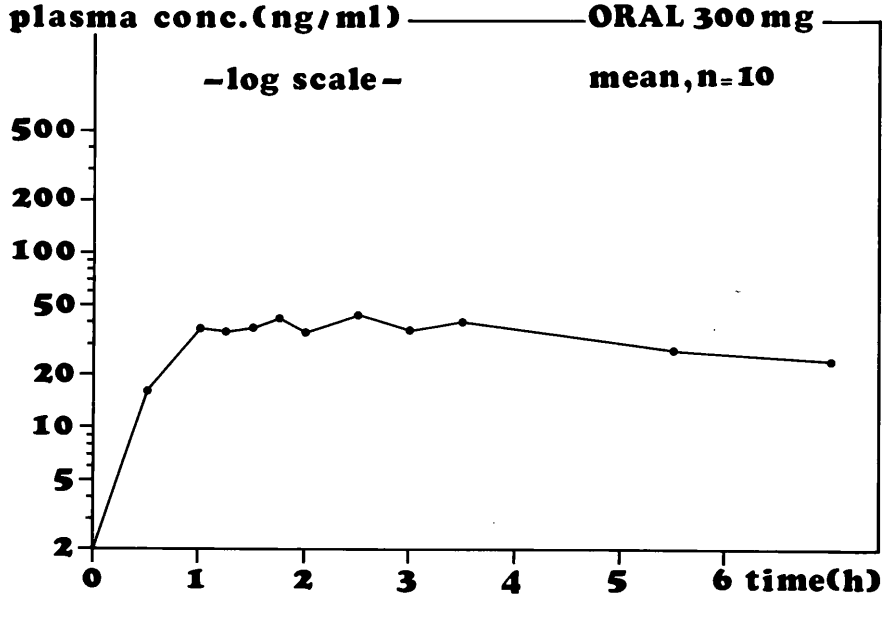


FIG. 4b: *The same data as in Fig. 4a, but drawn on semi-logarithmic scale.*

tinuing and that it was in equilibrium with elimination at that moment. An explanation for this phenomenon could be that the absorption process is biphasic: a rather rapid first phase followed by a much slower second one. The slower absorption in second instance can be understood by realizing that thiazinamium cations have a strong anticholinergic action; hence the motility of the intestines may be diminished by the drug (see Part I, Section 6.2.2.). Consequently absorption may be retarded and decreased as the local concentration of the drug in the intestines increases.
On the other hand, enterohepatic cycling and/or gastroenteral cycling could also be taken into account to explain the profile of the curves.
Fortunately, this long lasting plateau value will guarantee therapeutic plasma concentrations which lasts for several (at least 3 to 6) hours, if a sufficiently large dose is given. This is favourable during a therapeutic treatment.
Unfortunately, in almost all experiments the plasma concentrations were not followed long enough to allow determination of the individual biological half-life. Hence, it was impossible to estimate the lag-time and the half-life of absorption for the individual patients. As a measure for the rate of absorption, therefore, the time after which the maximum plasma concentration was reached t_{max} is given (see Table 1). The values for t_{max} varied from 60 to 210 min. with a mean value of 143 ± 50(S.D.) min.
The maximum plasma concentration varied from 33 to 126 ng/ml with a mean value of 73 ± 27 (S.D.) ng/ml.
From the plasma concentration-time curves during the time of the experiment (= 420 min.) a mean relative bioavailability F_{rel} of 13.9 ± 6.7 (S.D.) mg thiazinamium methylsulphate was calculated, which equals 0.034 ± 0.016 (S.D.) mmol or 3.6 ± 1.7 (S.D.)% of the dose (variation from 1.7 to 6.3% ; see Table 1). In a number of patients, the shape of the plasma concentration-time curve was such that no reliable extrapolation to concentration zero could be made.
In five patients, however, extrapolation could be performed and the total relative bioavailability (in Table 1 indicated as $(F_{rel})_{tot}$) could be estimated. In two patients (F.K. and J.A.M.) the curve declined to zero already during the experiment, so the $(F_{rel})_{tot}$ was measured. For these seven patients a mean value of 19.1 ± 8.3 (S.D.) mg of thiazinamium methylsulphate was found, which equals 0.049 ± 0.021 (S.D.) mmol or 4.9 ± 2.1 (S.D.) % of the dose. It should be realized, however, that the actual bioavailability was higher than these figures indicate, because the cases of better absorption are not incorporated in these mean values, extrapolation being impossible.
These figures of the bioavailability confirm the expectation that absorption of this quaternary ammonium compound is far from complete, and also that a substantial interindividual variation occurs. The latter point will be discussed in more detail in Section 4.3.3.

TABLE I: *Absorption characteristics of thiazinamium methylsulphate after oral administration of a dose of 300 mg in ten patients. (For symbols see Appendix 1).*

Patient		F.K.	J.A.M.	D.M.	D.H.	A.O.	O.V.	S.W.	K.W.	A.V.	E.D.	mean ± S.D.
Age	(years)	33	60	56	52	62	18	22	22	29	54	41 ± 18
Body weight	(kg)	78	88	71	64	82	83	66	68	71	69	74 ± 8
Height	(m)	1.78	1.83	1.68	1.73	1.67	1.82	1.83	1.90	1.80	1.67	1.77 ± 0.08
Dose*	(mg)	389.24	389.24	389.24	389.24	389.24	389.24	389.24	389.24	389.24	389.24	389.24
Dose*	(mg/kg)	4.99	4.42	5.48	6.08	4.75	4.69	5.90	5.72	5.48	5.64	5.32 ± 0.56
c_{max}	(ng/ml)	92	40	83	71	71	33	85	64	61	126	73 ± 27
t_{max}	(min.)	105	90	150	210	150	120	60	210	180	150	170 ± 96
F_{rel}	(mg)[1]	10.1	7.0	24.5	6.6	18.3	10.9	7.0	13.2	18.3	22.6	13.9 ± 6.7
F_{rel}	(% of dose)	2.6	1.8	6.3	1.7	4.7	2.8	1.8	3.4	4.7	5.8	3.6 ± 1.7
$(F_{rel})_{tot}$[2]	(mg)[1]	10.1	7.0	30.4	—	25.3	19.9	—	17.9	23.4	—	19.1 ± 8.3
$(F_{rel})_{tot}$[2]	(% of dose)	2.6	1.8	7.8	—	6.5	5.1	—	4.6	6.0	—	4.9 ± 2.1

[1] = expressed as mg of thiazinamium methylsulphate.
[2] = estimated values (except for patients F.K. and J.A.M.).
— = extrapolation of plasma concentration-time curve not possible.

4.3.2. Investigations with a single dose of 900 mg

Typical examples of plasma concentration-time curves are given in Fig. 5a and Fig. 5b. The curve for the averaged plasma concentrations for ten patients is represented in Fig. 6a and Fig. 6b. Most of the conclusions emerging from the 300 mg study were confirmed after giving a dose of 900 mg.

In most of the patients the curve rather rapidly reached a plateau level, which was maintained during several (3 to 6) hours. The maximum plasma concentration varied from 60 to 362 ng/ml with a mean value of 170 ± 96 (S.D.) ng/ml (see Table 2). In general this concentration was reached substantially later than after a 300 mg dose; t_{max} varied from 90 to 420 min. (mean 293 ± 135 (S.D.) min.). In four patients the curve was even going up at the last moment of the experiment. This delayed t_{max} after a 900 mg dose as compared to a 300 mg dose would seem to confirm that thiazinamium cations retard and probably diminish their own absorption.

From the plasma concentration-time curve a mean relative bioavailability F_{rel} of 52.3 ± 39.9 (S.D.) mg of thiazinamium methylsulphate was measured, which equals 0.128 ± 0.097 (S.D.) mmol or 4.5 ± 3.4 (S.D.) % of the dose. The interindividual variation was somewhat larger than after the 300 mg dose. At the end of the experiment, after 420 minutes, relatively high plasma plateau levels could be observed in a number of patients.

In five patients, however, the curve was distinctly descending and enabled extrapolation to zero concentration. In this way the total relative bioavailability (in Table 2 indicated as $(F_{rel})_{tot}$) in these patients could be estimated. A mean value of 108.6 ± 67.7 (S.D.) mg of thiazinamium methylsulphate was found, which equals 0.265 ± 0.165 (S.D.) mmol or 9.3 ± 5.8 (S.D.) % of the dose.

Although the variation in the total area under the plasma concentration-time curve was rather large, the variation in the maximum plasma concentrations was somewhat smaller.

Because absorption is far from complete, it is not amazing that a distinct variation occurs. In this respect, thiazinamium cations are comparable with other drugs with incomplete absorption, *e.g.* bishydroxycoumarine, digoxine and guanethidine (KOCH-WESER, 1974).

WILSON and others (1976) reported a large interindividual variation in plasma concentrations after single oral administration of propoxyphene and propranolol. For propoxyphene the highest observed value was 10 times the lowest observed value; in children, 20 times. For propranolol it was seven times. The authors suggested that this variation is due to "first pass effect" (see below). As we ourselves found indications which pointed towards a "first pass effect" during the absorption process of thiazinamium cations, the explanation of WILSON and associates is probably also valid

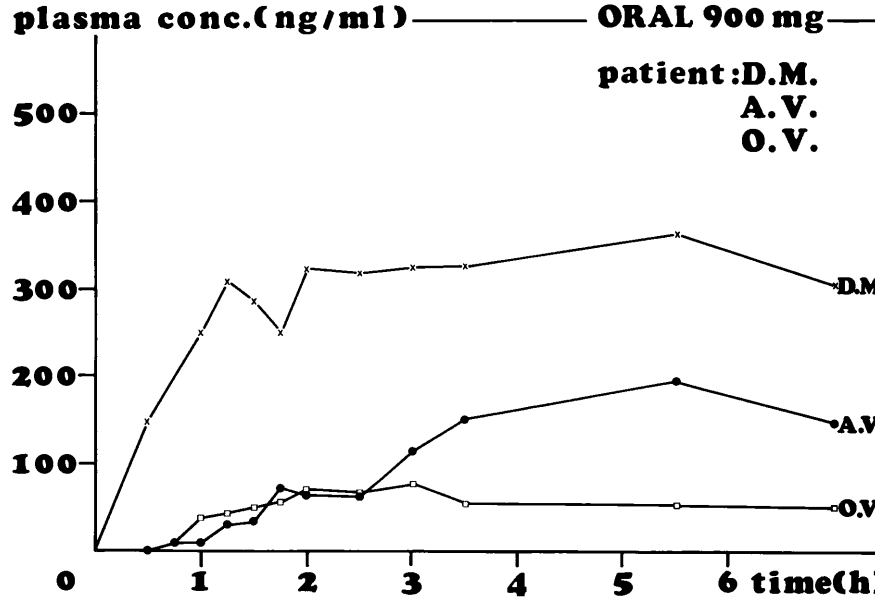


FIG. 5a : *Typical examples of individual plasma concentration-time curves obtained after oral administration of a dose of 900 mg. Linear scale.*

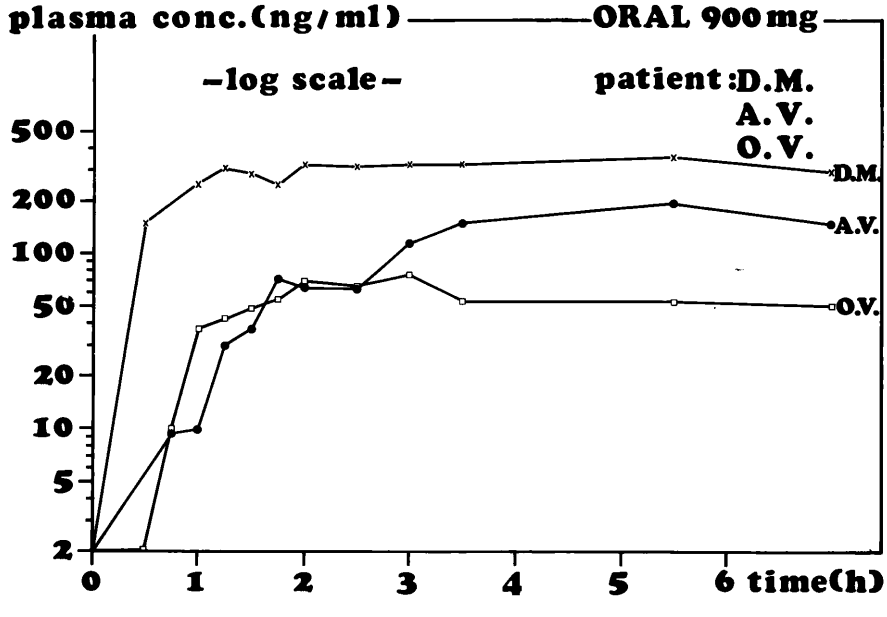


FIG. 5b: *The same data as in Fig. 5a, but drawn on semi-logarithmic scale.*

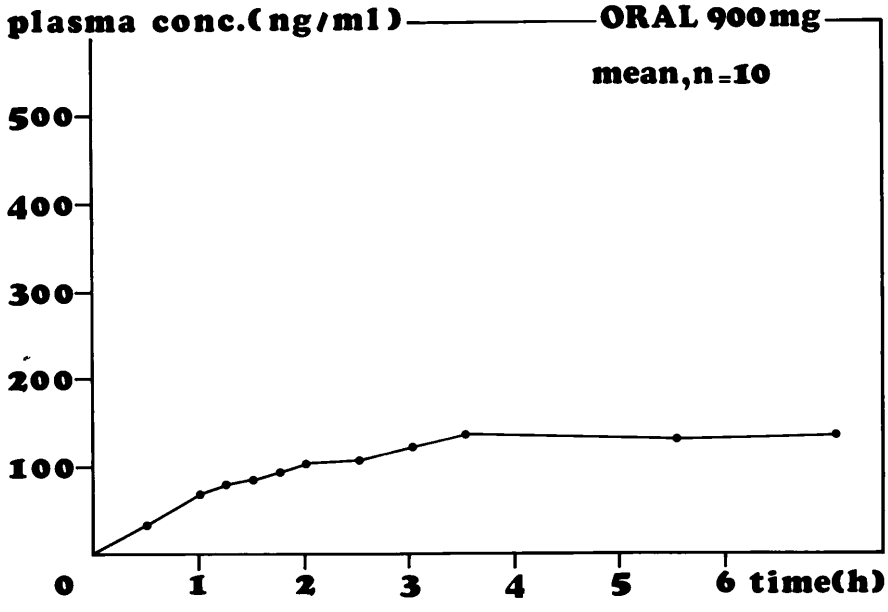


FIG. 6a: *Plasma concentrations (ng/ ml) after oral administration of a dose of 900 mg. The curve has been constructed from the averaged plasma concentrations of ten patients.*

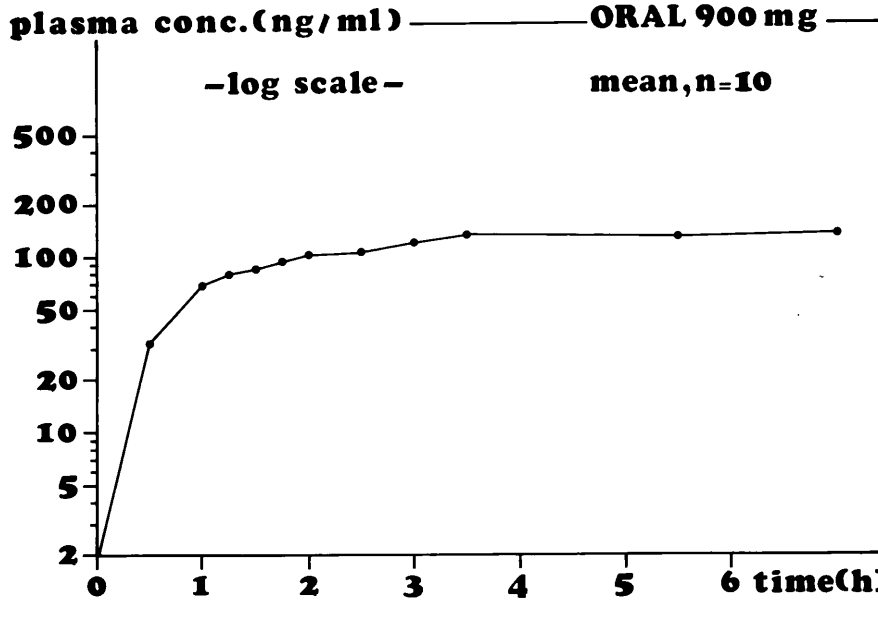


FIG. 6b: *The same data as in Fig. 6a, but drawn on semi-logarithmic scale.*

TABLE 2: *Absorption characteristics of thiazinamium methylsulphate after oral administration of a dose of 900 mg in ten patients. (For symbols see Appendix 1).*

Patient		F.K.	J.A.M.	D.M.	D.H.	A.O.	O.V.	S.W.	K.W.	A.V.	E.D.	mean ± S.D.
Age	(years)	33	60	56	52	62	18	22	22	29	54	41 ± 18
Body weight	(kg)	78	88	71	64	82	83	66	68	71	69	74 ± 8
Height	(m)	1.78	1.83	1.68	1.73	1.67	1.82	1.83	1.90	1.80	1.67	1.77 ± 0.08
Dose*	(mg)	1167.72	1167.72	1167.72	1167.72	1167.72	1167.72	1167.72	1167.72	1167.72	1167.72	1167.72
Dose*	(mg/kg)	14.97	13.27	16.45	18.25	14.24	14.07	17.69	17.17	16.45	16.92	15.95 ± 1.69
c_{max}	(ng/ml)	186	67	362	100	256	76	60	182	197	210	170 ± 96
t_{max}	(min.)	105	90	330	420	210	180	420	420	330	420	293 ± 135
F_{rel}	(mg)[1]	61.9	43.2	156.5	30.4	68.9	37.4	11.7	35.0	42.0	36.2	52.3 ± 39.9
F_{rel}	(% of dose)	5.3	3.7	13.4	2.6	5.9	3.2	1.0	3.0	3.6	3.1	4.5 ± 3.4
$(F_{rel})_{tot}$[2]	(mg)	74.7	60.7	213.7	—	140.1	—	—	—	53.7	—	108.6 ± 67.7
$(F_{rel})_{tot}$[2]	(% of dose)	6.4	5.2	18.3	—	12.0	—	—	—	4.6	—	9.3 ± 5.8

[1] = expressed as mg of thiazinamium methylsulphate.
[2] = estimated values.
— = extrapolation of plasma concentration-time curve not possible.

here. If so, "first pass effect" would be an additional cause of irregular bioavailability of thiazinamium cations.
We were surprised to find that in the majority of the cases the physiologically available amount of drug was not three times that found in the 300 mg experiment in the same individual. In some patients it was more but in other cases it was less. As this could also be explained by a substantial intraindividual variation in the absorption, we decided to study this phenomenon in more detail (see Section 4.3.3.).

If one compares the values found by us (see also JONKMAN and others, 1976 and 1977) for the relative bioavailability of thiazinamium methylsulphate with those reported in literature for other quaternary ammonium compounds, a good deal of similarity is seen, namely a mean value in the order of magnitude of 5 to 10% of the dose.
In this respect, however, it is of interest that we found several indications for a "first pass effect", which means that part of the thiazinamium cations are metabolized directly after they have been absorbed, before reaching the general circulation. In consequence, the values for the systemic bioavailability are *lower* than the amount of drug which was absorbed from the gastrointestinal tract.
Based on the amounts of unchanged drug and metabolite (= sulphoxide) in urine, the absorption was estimated to be approximately two times larger than the bioavailability (see Chapter 10).
In spite of their very low lipophilicity and their rather high molecular weight, then, thiazinamium cations can pass through the capillary membrane to a considerable degree. As the percentage of the dose absorbed after oral administration is substantially lower than the percentage absorbed after intramuscular administration (= 100%) this could be an indication that the water-filled intercellular pores in the capillary membrane in the gastrointestinal tract are smaller than those in the muscle. This would confirm HOGBEN's statement (1971) (see Introduction). Moreover, in the capillary bed of the muscle the contact will be direct; in the intestines, of course, it will not.
Apart from transport through the intercellular pores, other processes for gastrointestinal absorption cannot be excluded, but because of the poor lipophilicity (distribution coefficient n-octanol/water being appr. 0.015; see Part I, Section 2.8) passive diffusion of thiazinamium cations must be considered very unlikely.

4.3.3. Investigations with repeated doses of 900 mg

The results of this study showed that apart from a large interindividual variation there also existed a large intraindividual variation in the systemic availability of this quaternary ammonium compound (see Table 3). Among the volunteers, there seemed to be good and bad absorbers, but within each individual there was a difference in the amount of parent drug excreted, indicating the intraindividual variation. The mean systemic availability (from 0 to 24 hours) in this group of volunteers was 6.9 ± 4.0 (S.D.) % of the dose, which shows a fair correlation with the results of the previous studies in patients. The variations showed great differences among the volunteers. In some the variation was positively low (relative standard deviation about 20%), in others it was intermediate (30 to 40%) and in two subjects it was rather large (61 and 72%). The relative standard deviation from the mean in the whole group (71.0%) was larger than the relative standard deviation for the intraindividual variation (41.7%). This implies, that interindividual variation was more pronounced than intraindividual variation.

TABLE 3: *Amount of thiazinamium (Th) excreted in 24-hours' urine – expressed as mg of the methylsulphate – and total bioavailability F_{tot} after seven times a separate dose of 900 mg on different days in the same subject.*

Subject		B.D.	J.W.	R.A.Z.	H.J.G.G.	B.S.	R.E.G.	J.H.G.J.	G.J.E.
Th in 24-hours urine:									
mean	(mg)	8.1	8.9	9.2	17.0	17.1	22.3	24.3	39.3
S.D.	(mg)	5.0	2.7	4.3	4.5	3.5	16.1	8.8	14.9
relative S.D.	(%)	61.7	29.9	46.6	26.3	20.7	72.3	36.3	38.0
F_{tot} (% of the dose; estimated)		3.1	3.4	3.5	6.4	6.5	8.4	9.2	14.9

Conclusion

Thiazinamium cations, in spite of the relatively high molecular weight and the presence of a quaternary ammonium group, can be absorbed from the gastrointestinal tract. The exact mechanism (transport through pores by means of a "filtration process" or by means of "ion pair absorption") is not clear. Although the bioavailability is low, in the order of magnitude of 10%

of the dose, the plasma concentration-time curve shows a long lasting plateau value which ensures therapeutic plasma concentrations for several (at least 3 to 6) hours, provided that a sufficient dose was used. In bioavailability there was found substantial interindividual variation possibly due to variations in absorption and "first pass effect". Also intraindividual variation occurs. The relevance of the intraindividual variation is hard to estimate and comparison with intraindividual variation after administration of other drugs is almost impossible because literature on this subject is scarce.

In practice, however, the determination of plasma concentrations has proved to be helpful in establishing an optimal oral therapy for each patient.

References

ARIËNS, E. J. and F. A. NELEMANS (1975) in: *"Algemene Farmacotherapie"*, p. 672, (W. Lammers, F. A. Nelemans, Th. J. Bouwman, J. van Noordwijk and W. M. Rosinga, Eds.) Stafleu's Wetenschappelijke Uitgeversmaatschappij B.V., Leiden

BEERMANN, B., K. HELLSTRÖM and A. ROSÉN (1971a), *Clin. Sci. 40*, 95

BEERMANN, B., K. HELLSTRÖM and A. ROSÉN (1971b), *Europ. J. Clin. Pharmacol. 3*, 93

BEERMANN, B., K. HELLSTRÖM and A. ROSÉN (1971c), *Europ. J. Clin. Pharmacol. 4*, 46

BEERMANN, B., K. HELLSTRÖM and A. ROSÉN (1971d), *Acta Pharmacol. Toxicol. 29*, Suppl. 3, 98

BEERMANN, B., K. HELLSTRÖM and A. ROSÉN (1972a), *Europ. J. Clin. Pharmacol. 5*, 87

BEERMANN, B., K. HELLSTRÖM and A. ROSÉN (1972b), *Clin. Pharmacol. Ther. 13*, 212

BRODIE, B. B. (1964) in: *"Absorption and Distribution of Drugs"* (T. B. Binns, Ed.), E. & S. Livingstone Ltd., Edinburgh, London

CAVALLITO, C. J. and TH. B. O'DELL (1958), *J. Am. Pharm. Assoc. Sc. Ed., 47*, 169

COPER, H., G. DEYHLE and K. DROSS (1974), *Z. Klin. Chem. Klin. Biochem. 12*, 273

FIESE, G. and J. H. PERRIN (1969), *J. Pharm Sci. 58*, 599

GAGINELLA, T. S., P. BASS, J. H. PERRIN and J. J. VALLNER (1973a), *J. Pharm. Pharmacol. 25*, 270

GAGINELLA, T. S., P. BASS, J. H. PERRIN and J. J. VALLNER (1973b), *J. Pharm. Sci. 62*, 1121

GIBALDI, M. and B. GRUNDHOFER (1973), *J. Pharm. Sci. 62*, 343

HARINGTON, M. (1953), *Clin. Sci. 12*, 185

HELLSTRÖM, K., A. ROSÉN and K. SÖDERLUND (1970), *Scand. J. Gastroenterol. 5*, 585

HERXHEIMER, A. and L. HAEFELI (1966), *Lancet i*, 418

HOGBEN, C. A. M., L. S. SCHANKER, D. J. TOCCO and B. B. BRODIE (1957), *J. Pharmacol. Exp. Ther. 120*, 540

HOGBEN, C. A. M., D. J. TOCCO, B. B. BRODIE and L. S. SCHANKER (1959), *J. Pharmacol. Exp. Ther. 125*, 275

INNES, I. R. and M. NICKERSON (1975), in: "*The Pharmacological Basis of Therapeutics*", p. 523, (L. S. Goodman and A. Gilman, Eds.) MacMillan Publishing Co. Inc., New York, U.S.A.

IRWIN, G. M., H. B. KOSTENBAUDER, L. W. DITTERT, R. STAPLES, A. MISHER and J. W. SWINTOSKY (1969), *J. Pharm. Sci. 58*, 313

JONKMAN, J. H. G., L. E. VAN BORK, J. WIJSBEEK, R. A. DE ZEEUW and N. G. M. ORIE (1976), *Pharm. Weekblad 111*, 1209

JONKMAN, J. H. G., L. E. VAN BORK, J. WIJSBEEK, R. A. DE ZEEUW and N. G. M. ORIE (1977), *Clin. Pharmacol. Ther., 21*, 457

KOCH-WESER, J. (1974), *New Engl. J. Med. 291*, 233

KONDRITZER, A. A., P. ZVIRBLIS, A. GOODMAN and S. H. PAPLANUS (1968), *J. Pharm. Sci. 57*, 1142

KUNZE, H., K. BLINNE and W. VOGT (1971), *Naunyn-Schmiedebergs Arch. Exptl. Pathol. Pharmak. 270*, 161

LEVINE, R. R. (1961), *J. Pharmacol. Exp. Ther. 131*, 328

LEVINE, R. R. (1966), *Arzneimittel-Forsch. 16*, 1373

LEVINE, R. R., M. R. BLAIR and B. B. CLARK (1955), *J. Pharmacol. Exp. Ther. 114*, 78

LEVINE, R. R. and E. W. PELIKAN (1961), *J. Pharmacol. Exp. Ther. 131*, 319

LEVINE, R. R. and E. W. PELIKAN (1964), *Ann. Rev. Pharmacol. 4*, 69

LEVINE, R. R. and A. F. SPENCER (1961), *Biochem. Pharmacol. 8*, 248

MASAKI, B. W., E. J. LIEN and J. A. BILES (1973), *Acta Pharm. Suecica 10*, 43

MROSZCZAK, E. J., J. J. VALLNER and J. H. PERRIN (1969), *J. Pharm. Sci. 58*, 1567

PERRIN, J. H. and J. J. VALLNER (1970), *J. Pharm. Pharmacol. 22*, 758

PFEFFER, M., J. M. SCHOR, S. BOLTON and R. JACOBSEN (1968a), *J. Pharm. Sci. 57*, 1375

PFEFFER, M., J. M. SCHOR, N. GLUCK, M. G. SEMMEL and S. GRIBOFF (1968b), *J. Pharm. Sci. 57*, 36

PLAKOGIANNIS, F. M., E. J. LIEN, C. HARRIS and J. A. BILES (1970), *J. Pharm. Sci. 59*, 197

RITSCHEL, W. A. (1976), in: *"Handbook of Basic Pharmacokinetics"*, Drug Intelligence Publications, Hamilton, Illinois 62341, U.S.A.

SCHANKER, L. S. (1962), *Pharmacol. Rev. 14*, 501

SCHANKER, L. S. (1971) in: *"Concepts in Biochemical Pharmacology"*, Part 1, p. 9 (B. B. Brodie and J. R. Gilette, Eds.), Springer Verlag, Berlin, New York

SIDELL, F. R. and W. A. GROFF (1969), *J. Pharm. Sci. 58*, 1093

SIDELL, F. R. and W. A. GROFF (1971), *J. Pharm. Sci. 60*, 860

SIDELL, F. R., W. A. GROFF and A. KAMINSKIS (1972), *J. Pharm. Sci. 61*, 1137

SUNDWALL, A. (1961), *Biochem. Pharmacol. 5*, 225

SUNDWALL, A., J. VESSMAN and B. STRINDBERG (1973), *Europ. J. Clin. Pharmacol. 6*, 191

TOMLINSON, E. and S. S. DAVIS (1976), *J. Pharm. Pharmacol. 28*, Suppl. 75P

UNITED STATES PHARMACOPEIA XIX (1975), The United States Pharmacopeial Convention Inc., Rochville, Md.

WAGNER, J. G. (1971) in: *"Biopharmaceutics and Relevant Pharmacokinetics"*, Drug Intelligence Publications, Hamilton, Illinois 62341, U.S.A.

WILSON, J. T., G. T. ATWOOD and D. G. SHAND (1976), *Clin. Pharmacol. Ther. 19*, 264

5

Bioavailability after rectal administration in man

Contents

5.1. Introduction

Rectal administration is not only used for local activity but it is also in use for several classes of drugs with systemic action, for example analgetics, spasmolytic drugs and drugs against generalized obstructive lung diseases (*e.g.* theophylline derivatives). This route of administration is recommended when the oral route is precluded by vomiting, when the patient is unconscious, for drugs which are likely to cause gastric irritation, and whenever one wants to obtain a rather rapid effect by means of a noninvasive method.

Several textbooks on therapeutics indicate that after rectal administration drugs will enter – at least to a large extent – the general circulation without initial passage through the liver, provided that the suppository does not reach the higher parts of the rectum (*e.g.* GOODMAN and GILMAN, 1975; LAMMERS and others, 1975; see also BUCHER, 1948; HENNIG, 1959; GREENLEAF and HADGRAFT, 1960). The drug would come into the *vena rectalis caudalis* (= inferior rectal vein) and/or *vena rectalis medialis* (= middle rectal vein), which drain into the *vena cava inferior*. On the other hand, the

blood in the *vena rectalis cranialis* (= superior rectal vein) flows to the *vena porta* (= portal vein) and subsequently enters the liver (Fig. 1).

BUCHER (1948) found that when a suppository, containing Na_2HPO_4, is placed into the lower sixth of the rectum of the rat, about 75% of the amount absorbed passes directly into the systemic circulation. But even if the suppository is in contact with the lower two-thirds of the rectum, the amount bypassing the portal system exceeds 50 percent.

HENNIG (1959) found on quinine and on sodium para-aminosalicylate that only 12 to 20% of the dose passes the liver before entering the peripheral venous circulation.

It is common knowledge that after *oral* administration most drugs enter the *vena porta*, which precedes a 100 percent passage through the liver before entering the general circulation.

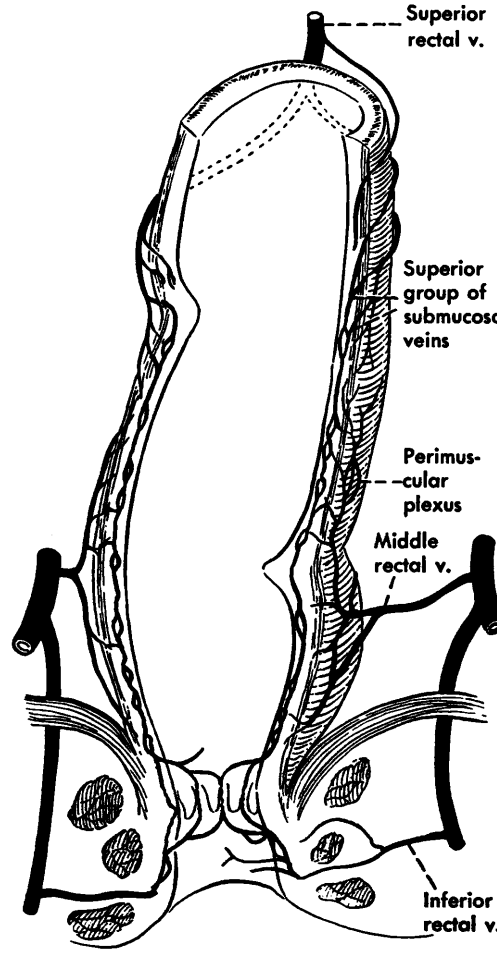


FIG. 1: *Scheme of the venous drainage of the rectum. (From* HOLLINSHEAD, *1961).*

Once it has entered the general circulation, approximately 20% of the total blood flow passes through the liver during each circulation, whatever the route of administration.
When drugs are administered by a route other than the oral one, for example parenteral or rectal, the complete first passage through the liver does not occur.
For this reason rectal administration would also be recommended as a noninvasive alternative for those drugs that are in large part metabolized by the liver or excreted in the bile, and for drugs which are subjected to degradation in the gastrointestinal tract.

In an excretion and metabolism study (Chapter 10), we found clear indications that an extensive "first pass effect" occurs when thiazinamium cations are administered orally. Moreover, a high proportion of both the unchanged drug and the metabolite are found in the bile (see Chapter 9). For these reasons we decided to investigate if rectal administration of this drug would entail better bioavailability.
The relative bioavailability of rectally administered thiazinamium methylsulphate as compared to intramuscular injection was investigated using two different kinds of suppositories, one with a lipophilic base and one with a hydrophilic base. This was done to gain an insight into the optimum composition of suppositories containing thiazinamium methylsulphate. The optimum formulation of these suppositories should meet two prerequisites: it must give good bioavailability and it must cause minimal local irritation. The latter factor is important because it is known from practice that suppositories containing thiazinamium methylsulphate in cacao-butter can cause substantial local irritation, often resulting in premature expulsion of the suppository. Although many drugs cause irritation of the rectal mucosa (FINGL and WOODBURY, 1975), this phenomenon seems to be rather pronounced for thiazinamium methylsulphate, possibly due to the quaternary ammonium group which may be suspected to have surface-active properties.
In a second study we investigated if the concept of "ion pair absorption" applies to some thiazinamium ion pairs after rectal administration. As discussed in detail in Section 4.1., several investigators have found that absorption (or in most cases disappearance from the gastrointestinal tract) of quaternary ammonium compounds could be increased by adding a counter ion of ion pair forming potential. Absorption of the quaternary ammonium ion, then, is supposed to occur as a neutral, more lipophilic, ion pair. However, we criticized this theory, because in general, ion pairs still have some water solubility (see Part II, Chapters 1 and 2). After oral application in man, the ion pair will come into a rather large volume of fluids (several hundreds of ml). This large volume favours dissociation of the ion pair and hence reduces the amount of ion pairs available for

diffusion through the membrane. In order to counteract this effect, most investigators added large amounts (up to a molecular ratio of 1 : 90) of excess counter ions, but this can result in adverse effects of the counter ions, which are mostly of exogenous nature.

In the rectum, however, the volume of liquid is considerably smaller: it contains 1 to 3 ml of viscous fluids (BEVERNAGE and POLDERMAN, 1973; RITSCHEL, 1973). This led us to assume that the degree of dissociation of ion pairs in this small volume of fluids will be low, and hence, if any dissociation does occur it can possibly be reduced by relatively small amounts of excess counter ions. We therefore studied the bioavailability after rectal administration of thiazinamium iodide and thiazinamium salicylate alone, and with an excess of counter ions in a molecular ratio of 1 : 5.

TABLE I: *Solubility of thiazinamium as methylsulphate, iodide and salicylate in aqueous solutions (pH = 1.2 and 7.4) and in n-octanol. (Data obtained from M. BENTEJAC, SPECIA, Rhône Poulenc, Paris, France).*

solvent	solubility (mg/ml)		
	thiazinamium methylsulphate	thiazinamium iodide	thiazinamium salicylate
aqueous solution (pH = 1.2)	85.0	0.2	8.8
aqueous solution (pH = 7.4)	48.0	0.1	10.2
n-octanol	0.7	0.3	28.4

Table 1 represents the solubilities of some thiazinamium derivatives in aqueous solution of pH = 1.2 and pH = 7.4 and in n-octanol.

5.2. Materials and methods

5.2.1. Investigations with Witepsol H-15 suppositories

Subjects and conditions

The patients as described in Section 3.2.2.2. again participated in this trial. The time schedule for blood sampling and voiding urine, and the other conditions of the experiment were identical to those described there.

The dosage form

All dosage forms for rectal application were prepared by DRS. H. L. M. COX at the Laboratorium der Nederlandse Apothekers (= L.N.A., Laboratory of the Dutch Pharmacists) at The Hague.
As a *lipophilic (fatty)* base Witepsol H-15 (= WH-15) was selected (Dynamit Nobel A.G./ Chemische Werke Witten, Witten, G.F.R.). Witepsol H-15 is a mixture of mono-, di- and triglycerides of naturally occurring saturated fatty acids (C_{12} to C_{18}), with a melting range of 33.5 to 35.5° C and a congealing range of 32.5 to 34.5° C. The specific gravity of Witepsol H-15 at 20° C is 0.950 to 0.980; it has an iodine number less than 3, a saponification value of 230 to 240 and a hydroxyl value less than 15 (see the leaflet "WITEPSOL® FÜR SUPPOSITORIEN", Dynamit Nobel A.G./Chemische Werke Witten, Witten, G.F.R., 1967). Its melting behaviour, both *"in vitro"* and *"in vivo"*, is described by RITSCHEL (1973), who showed that a suppository of Witepsol H-15 without additional drug substances melts almost completely in the human rectum within 10 min.
For all experiments with rectal administration of the drug, a dose of 0.4747 mmol = 150 mg of thiazinamium base (= hydroxyde) was selected, which equals 194.62 mg of the methylsulphate. The substance was passed through a sieve in order to obtain particles < 150 µm in size. Then 2 mg of colloidal silica (Aerosil®) per suppository was added and mixed with the drug substance. The reason for adding colloidal silica is explained below. Suppositories were prepared by making a suspension of the powder in the molten base (about 50° C), after which the liquid was poured into 2-ml moulds. More details of the method of preparing such suppositories can be found in the "FORMULARIUM DER NEDERLANDSE APOTHEKERS".
The content of drug substance in the suppositories was determined by COX (Laboratory of the Dutch Pharmacists), by means of amphimetric titration. For this the method described in Part I, Section 4.7.2. was used in a modified form. The suppository was dissolved in 45 ml of chloroform and titrated as

described in that section (Differences: Acetic acid / sodium acetate buffer of pH = 2.8; the indicator was a mixture of 3 mg oracet blue and 3 mg of dimethyl yellow in 100 ml of chloroform; 0.01 molar D.O.S.S. was used.). The content of the suppositories was found to be 100.3% (n = 3) of the stated amount.

The dissolution rate of the suppositories was determined by the Laboratory of the Dutch Pharmacists making use of a method developed by COX. This method uses boiled water as dissolution medium, but does not use a membrane. Within 30 min. an amount of 83% of the content of the suppositories appeared in the aqueous phase (Fig. 2).

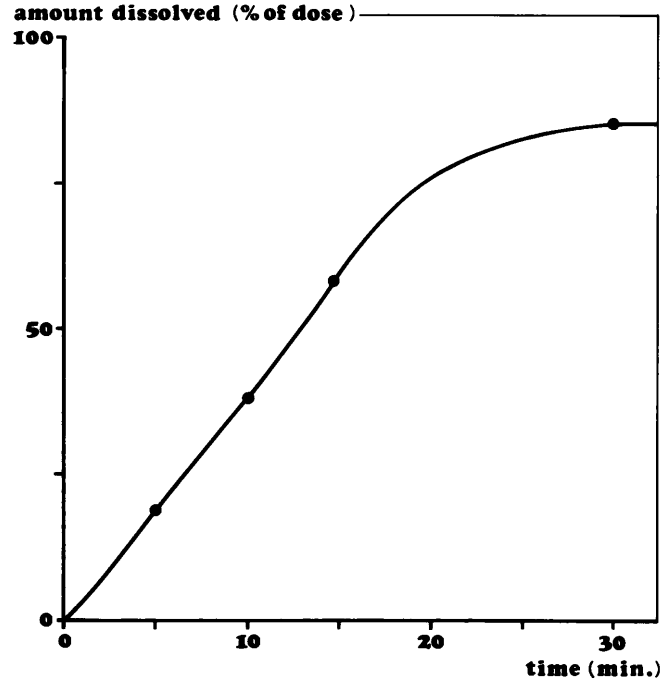


FIG. 2: *Release of thiazinamium methylsulphate from a Witepsol H-15 suppository.*

Determination of thiazinamium cations in plasma

The amount of drug in plasma was determined as described in Part II, Section 2.3 and expressed as nanograms of thiazinamium methylsulphate per ml of plasma.

Determination of the relative bioavailability

The areas under the plasma concentration-time curve were determined by cutting and weighing of a standard type of high quality paper. The relative bioavailability *versus* an intramuscular injection of a dose of 12.5 mg was calculated as described in Chapter 1.

5.2.2. Investigations with polyethylene glycol suppositories

Subjects and conditions

The patients were the same as in the previous section.

The dosage form

As a *hydrophilic* or *polar* base a polyethylene glycol (= PEG) mixture was selected (Union Carbide Corporation, New York, U.S.A.). The trade mark is Carbowax®. The mixture consists of PEG 1500 and PEG 4000 in a ratio of 1:2. PEG 1500 is a blend of equal parts of PEG 300 and 1540 and it has the consistency of petrolatum. PEG 4000 is a white waxy solid. From a chemical point of view, polyethylene glycols are polymers of ethylene oxide with the generalized formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, where n represents the average number of oxyethylene groups. The melting range of PEG 1500 is 38.0 to 41.0° C, of PEG 4000 it is 53.0 to 56.0° C. The specific gravity of PEG 1500 at 20/20° C is 1.151, of PEG 4000 it is 1.204 (density g/ml). The PEG suppositories were prepared as follows. The powder mixture of thiazinamium methylsulphate and colloidal silica was prepared as described in the previous section. The PEG was liquefied at 80° C and mixed with the powder; it was then pourced at 50° C into 2-ml moulds.
The content of the drug substance in PEG suppositories could not be determined by means of an amphimetric titration because the PEG mixture itself consumes a considerable amount of D.O.S.S. (about 9 ml/suppository). For that reason direct spectrophotometric analysis as described in Part I, Section 4.4. was carried out here. The content of the suppositories was found to be 103.2% of the stated amount.
The dissolution rate of PEG suppositories could not be determined according to the above method because the method is only applicable to lipophilic suppositories.
N.B. In order to avoid the influence of the pharmaceutical phase (= desintegration of dosage form, dissolution of active substance) preferably rectioles containing an aqueous solution of thiazinamium methylsulphate

should be integrated in this study. However, we expected strong local irritation and we decided to omit this dosage form.
All other conditions, materials and methods were identical to those described in the previous section.

5.2.3. "Ion pair absorption" studies

Subjects and conditions

The subjects were female or male volunteers, mainly medical students, of about 25 years. Subjects were not suffering from any known illness. They had been fasting overnight prior to the experiments. The suppository was applied at about 07.00 a.m. They stayed in bed during the first 20 min. after administration of the drug. About one hour after drug administration they ate breakfast, after which normal daily activities were allowed; food and liquid intake were *ad libitum*. The amount of drug which had been available in the body was monitored by determining the excretion of the parent drug in the urine collected during 24 hours. In this "screening test" the various dosage forms were administered on different days according to a double blind randomized cross over trial in a Latin square design (each subject receiving each formulation) (WESTLAKE, 1973). Between the individual experiments there was a "wash out" period of at least two days.

The dosage form

For the *"ion pair absorption" screening test* two ion pairs of thiazinamium cation were under investigation, *viz.* the iodide and the salicylate, both kindly supplied by SPECIA, Rhône Poulenc, Paris, France. The same vehicles, namely WH-15 and PEG were used in this study. This experiment consisted of four different tests, in each of which five dosage forms were investigated, namely *1.* the vehicle without any drug substance (= "blank"), *2.* thiazinamium methylsulphate (for comparison), *3.* the ion pair, *4.* the ion pair with an excess of ion pair forming counter ions (molecular ratio of 1 : 5), and *5.* thiazinamium methylsulphate with the excess of counter ions, in order to achieve *"in vivo"* ion pair formation. As counter ion iodide was added as potassium iodide and salicylate as sodium salicylate. For further details see Table 2. Microscopic analysis of the drug substances showed that the powder of thiazinamium iodide and thiazinamium salicylate consisted merely of small crystalls (particle size 4–8 μm), but that a notable quantity of larger agglomerates also occurred. These agglomerates could be disintegrated by adding a small amount of colloidal silica : 2.0 mg (= 0.1%)

TABLE 2: *Composition of the suppositories used in the "ion pair absorption" screening test.*

	particle size (μm)	content (% of stated amount)
Group A: base Witepsol H-15		
1. blank	—	—
2. thiazinamium methylsulphate (ThMS)	< 150	103.3
3. thiazinamium iodide (ThI)	4–8[1]	102.6
4. thiazinamium methylsulphate + excess of potassium iodide, molecular ratio 1 : 5 (ThMS/KI)	resp. < 150 and < 100	123.8
5. thiazinamium iodide + excess of potassium iodide, molecular ratio 1 : 5 (ThI/KI)	resp. < 150 and < 100	120.2
Group B: base polyethylene glycol 1500/4000 (1 : 2)		
1. blank	—	—
2. thiazinamium methylsulphate (ThMS)	150	103.2
3. thiazinamium iodide (ThI)	4–8[1]	98.5
4. thiazinamium methylsulphate + excess of potassium iodide, molecular ratio 1 : 5 (ThMS/KI)	resp. < 150 and < 100	104.9
5. thiazinamium iodide + excess of potassium iodide, molecular ratio 1 : 5 (ThI/KI)	resp. 4–8[1] and < 100	107.4
Group C: base Witepsol H-15		
1. blank	—	—
2. thiazinamium methylsulphate (ThMS)	< 150	100.3
3. thiazinamium salicylate (ThSal)	4–8[1]	102.7
4. thiazinamium methylsulphate + excess of sodium salicylate, molecular ratio 1 : 5 (ThSal/NaSal)	resp. < 150 and < 100	102.4
5. thiazinamium salicylate + excess of sodium salicylate, molecular ratio 1 : 5 (ThSal/NaSal)	resp. 4–8[1] and < 100	102.7
Group D: base polyethylene glycol 1500/4000 (1 : 2)		
1. blank	—	—
2. thiazinamium methylsulphate (ThMS)	< 150	95.8
3. thiazinamium salicylate (ThSal)	4–8[1]	92.1
4. thiazinamium methylsulphate + excess of sodium salicylate, molecular ratio 1 : 5 (ThMS/NaSal)	resp. < 150 and < 100	96.6
5. thiazinamium salicylate + excess of sodium salicylate, molecular ratio 1 : 5 (ThSal/NaSal)	resp. 4–8[1] and < 100	102.8

[1] = see also text.

per suppository proved to be effective. This low quantity of colloidal silica did not influence the viscosity of the suppositories. In order to obtain comparable dosage forms this amount of colloidal silica was added to all kinds of suppositories. The suppositories were prepared as described in Sections 5.2.1. and 5.2.2.
All other conditions, materials and methods were identical to those described in the previous section.

5.3. Results and discussion

5.3.1. Investigations with Witepsol H-15 suppositories

Typical examples of plasma concentration-time curves are given in Fig. 3a (linear scale) and Fig. 3b (semi-logarithmic scale). The curve for the averaged plasma concentrations for seven patients is represented by Fig. 4a and by Fig. 4b.

Plasma concentrations were generally found to be low. In most of the patients it appeared that absorption started rather soon. The values for t_{max} varied from 15 to 120 min. with a mean value of 63 ± 42 (S.D.) min. (see Table 3). Due to the fact that most of the individual curves have their maximum at different points in time between t = 15 and t = 120 min. the curve of the average plasma concentrations-time is almost horizontal during this period and even shows two less pronounced maxima. It can therefore be considered as a typical example of a mean curve which gives misleading information, which confirms the statements of VAN ROSSUM (1976) and BREIMER (1974) (see also the remarks made about this subject in Section 4.3.1.).
The rather low values for t_{max} suggest that thiazinamium cations are readily liberated from the WH-15 base in the rectum. This is in agreement with the *"in vitro"* experiment (see Fig. 2). It also agrees with the general rule, that water-soluble (hydrophilic) compounds, which do not dissolve in a fatty (lipophilic) base, are rapidly released by the base after it has melted (see *e.g.* HENNIG, 1959; MÜNZEL, 1966; KERCKHOFFS and HUIZINGA, 1967; BEVERNAGE and POLDERMAN, 1973; RITSCHEL, 1973).
The values for c_{max} varied from 54 to 290 ng/ml, with a mean value of 103 ± 89 (S.D.) ng/ml. However, it should be noticed that this mean value is

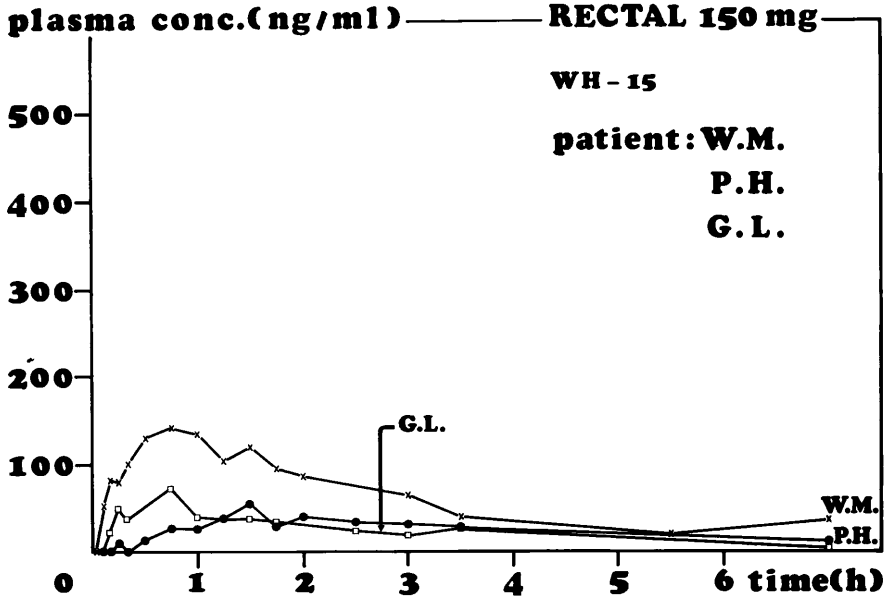


FIG. 3a: *Typical examples of individual plasma concentration-time curves obtained after rectal administration of a Witepsol H-15 suppository containing a dose of 150 mg. Linear scale.*

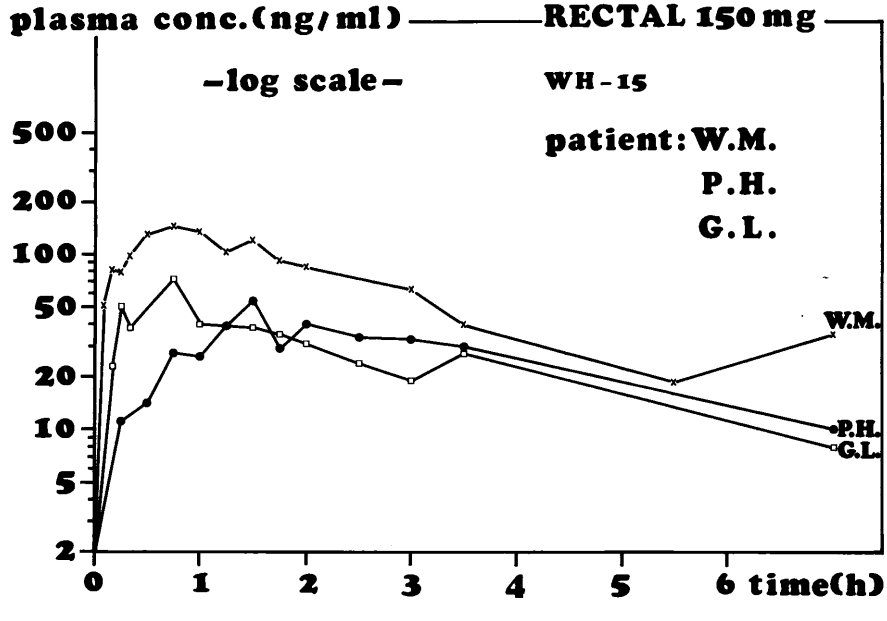


FIG. 3b: *The same data as in Fig. 3a, but drawn on semi-logarithmic scale.*

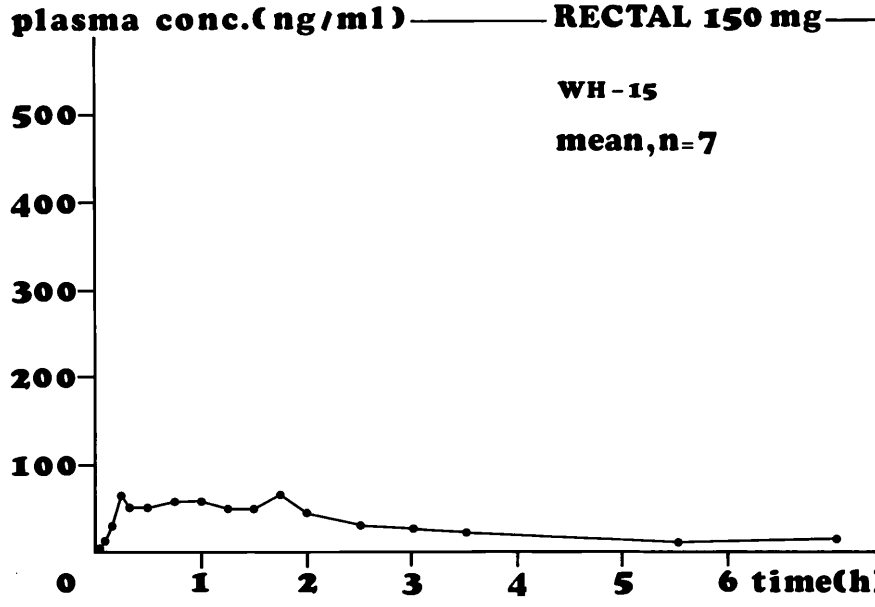


FIG. 4a: *Plasma concentrations (ng/ml) after rectal administration of a Witepsol H-15 suppository containing a dose of 150 mg. The curve has been constructed from the averaged plasma concentrations of seven patients.*

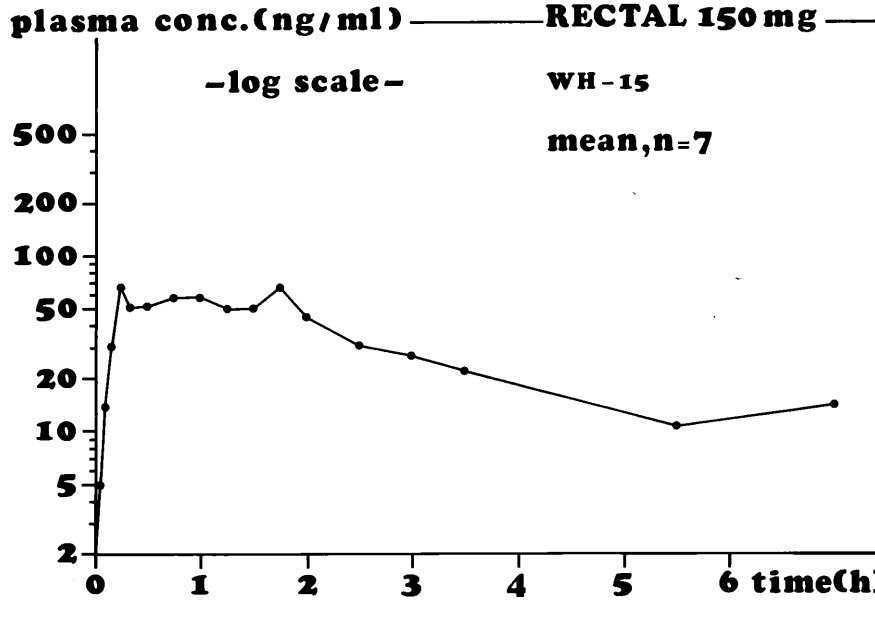


FIG. 4b: *The same data as in Fig. 4a, but drawn on semi-logarithmic scale.*

TABLE 3: *Absorption characteristics of thiazinamium methylsulphate after rectal administration of a dose of 150 mg. (Vehicle: Witepsol H-15).*

Patient		G.L.	P.H.	W.M.	H.H.	W.B.	M.F.	T.J.	mean ± S.D.
Age	(years)	57	62	52	63	26	71	68	57 ± 15
Body weight	(kg)	74	86	79	67	70	72	75	75 ± 6
Height	(m)	1.78	1.74	1.81	1.76	1.74	1.76	1.77	1.77 ± 0.02
Dose*	(mg)	194.62	194.62	194.62	194.62	194.62	194.62	194.62	194.62
Dose*	(mg/kg)	2.63	2.26	2.46	2.90	2.78	2.70	2.59	2.62 ± 0.21
c_{max}	(ng/ml)	72	54	142	63	45	290	53	103 ± 89
t_{max}	(min.)	45	90	45	120	105	15	20	63 ± 42
F_{rel}	(mg)[2]	12.2	12.9	21.3	13.4	1.3	11.3	8.0	11.5 ± 6.0
F_{rel}	(% of dose)	6.3	6.6	11.0	6.9	0.7	5.8	3.4	5.8 ± 3.2
$(F_{rel})_{tot}$[1]	(mg)[2]	12.8	13.2	22.6	15.8	1.3	11.3	7.2	12.0 ± 6.7
$(F_{rel})_{tot}$[1]	(% of dose)	6.6	6.8	11.6	8.1	0.7	5.8	3.7	6.2 ± 3.4

[1] = estimated values, except for patient W.B. and M.F. (measured values).
[2] = expressed as mg of thiazinamium methylsulphate.

much affected by the high values found in subjects W.M. and M.F. These two being omitted, the remaining five data varied from 45 to 72 ng/ml (mean 57 ± 10 (S.D.) ng/ml).

Thus, as compared to oral administration, the absorption process after rectal application began earlier and seemed to take place at a higher rate, even if a 15 to 30 min. period was taken into account for passage through the stomach. Moreover, after rectal application the peak is more pronounced. Although the rectally given dose is only half of that given orally, the peak concentrations are in the same order of size.

In four patients the curve after reaching c_{max} declines rather fast resulting in a concentration of zero or almost zero ng/ml at the end of the experiment (t = 420 min). Here the shape of the curve is intermediate between those generally seen after oral administration and those which are seen after intramuscular administration. But the profiles are somewhat irregular and hardly enable reliable pharmacokinetic analysis. In these patients the biological half-life $(t_{\frac{1}{2}})_{\beta}$ could roughly be estimated and appeared to be between 150 and 250 min., which is in the same order of magnitude as was often found in other patients after intramuscular injection of the drug.

However, in three other patients a more or less pronounced plateau level was found, but it did not stretch out over such a large period as after oral

administration. This may indicate, that absorption of the drug goes on for a rather extended period, despite the fact that it is readily released from the base. This finding can probably be explained by the fact that the rectum does not have any motility, which results in a long period of contact between the drug substance and the membrane to be passed, although it is not clear - in spite of the absence of motility - to what extent the molten base reaches higher parts of the rectum. In this respect the results obtained in patient H.H. are of interest. This patient was the only one who reported a substantial local irritation, resulting in defecation 45 min. after application. The patient reported that part of the suppository was lost during the evacuation. Until that moment no plasma concentration was found, but after this defecation absorption started rapidly and resulted in a plateau level. This suggests that part of the drug was absorbed from the molten base in the higher parts of the rectum. Similar findings in patient G.L. support this assumption. This patient reported irritation and lost his suppository after 20 min., but absorption continued to take place after defecation. Two of the other patients reported slight, but tolerable irritation between 10 to 20 min. after application.

From the plasma concentration-time curves during the time of the experiments (= 420 min.) a mean relative bioavailability F_{rel} of 11.5 ± 6.0 (S.D.) mg thiazinamium methylsulphate was calculated which equals 0.028 ± 0.015 (S.D.) mmol or 5.8 ± 3.2 (S.D.) % of the dose (range: 0.7 to 11.0%; see Table 3). In five patients extrapolation was possible, which led to an estimated total relative bioavailability $(F_{rel})_{tot}$ of 7.4 ± 2.9 (S.D.) % of the dose. In two patients, (W.B. and M.F.) the curve declined already to zero during the time of the experiment, so the $(F_{rel})_{tot}$ was measured; for all seven patients $(F_{rel})_{tot}$ was 6.2 ± 3.4 (S.D.) % of the dose. These bioavailability values are in the same order of magnitude as after oral administration.

This is rather amazing because there occur no villi in the rectum and hence the surface of the membrane, and consequently the number of pores in it, is lower than in the small intestines.

The results of our study suggest that rectal administration also resulted in a considerable interindividual variation. Because we found indications that a "first pass effect" also occurs after rectal application (see Chapter 11) this might be - in part - an explanation of this variation.

KERCKHOFFS and HUIZINGA (1967) concluded from the results of a similar study that absorption after rectal administration of thiazinamium methylsulphate is more rapid, but beside that, also more complete than absorption after giving by the oral route (see, however, Part II, Chapter 2). SUNDWALL and others (1971) compared oral and rectal administration (WH-15 suppositories) of the quaternary ammonium compound emepronium bromide in dog, and found almost identical bioavailability after these routes.

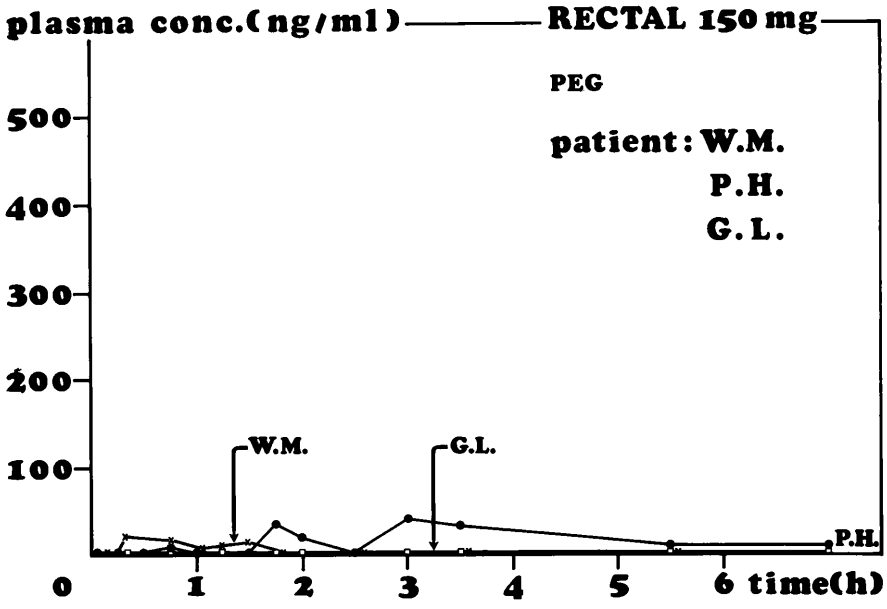


FIG. 5: *Typical examples of individual plasma concentration-time curves obtained after rectal administration of a polyethylene glycol suppository (PEG 1500/4000, ratio 1:2) containing a dose of 150 mg. Linear scale.*

5.3.2. Investigations with polyethylene glycol suppositories

Plasma concentrations achieved after application of the PEG suppositories were extremely low. In one patient (G.L.) no drug substance could be detected at all in the plasma. In the other patients no thiazinamium cations could be detected in a number of samples. If any drug could be determined it was at irregular times and often close to the detection limit. Typical examples of plasma concentration-time curves are given in Fig. 5. The curve for the averaged plasma concentrations for seven patients is represented by Fig. 6. These findings suggest that thiazinamium cations became available in the rectal fluid at a low rate and in a small proportion. This is in agreement with the general rule that water-soluble (= hydrophilic) compounds are liberated slowly from a hydrophilic base like PEG. This may be due to the fact that the dissolution time of PEG suppositories is long in comparison to the melting time of WH-15 suppositories; the former may lie in the range of one hour or more (HENNIG, 1959; BEVERNAGE and POLDERMAN, 1973). KERCKHOFFS and HUIZINGA (1967) studied *"in vitro"* dissolution time of suppositories containing thiazinamium methylsulphate in the same PEG mixture and found a release of about 60% in four hours.

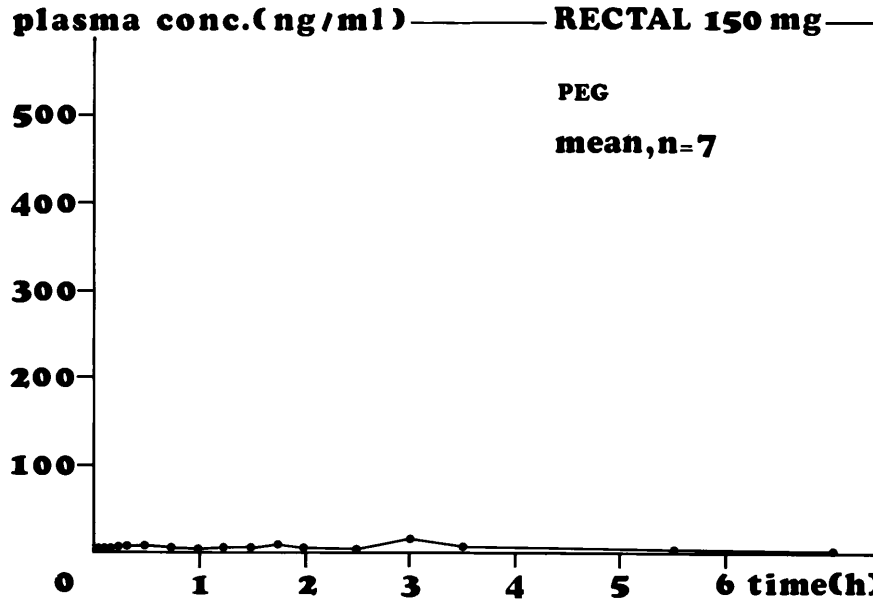


FIG. 6: *Plasma concentrations (ng/ml) after rectal administration of polyethylene glycol suppository (PEG 1500/4000, ratio 1:2) containing a dose of 150 mg. The curve has been constructed from the averaged plasma concentrations of seven patients.*

Apparently, if any absorption of thiazinamium cations from PEG suppositories does at all occur, the elimination rate is almost the same or even higher than the absorption rate. Hence, a relative bioavailability F_{rel} was calculated of 0.8 ± 1.1 (S.D.)% of the dose (Table 4).

TABLE 4: *Absorption characteristics of thiazinamium methylsulphate after rectal administration of a dose of 150 mg (Vehicle: PEG 1500/4000 (1 : 2)). For patient characteristics see Table 3.*

		G.L.	P.H.	W.M.	H.H.	W.B.	M.F.	T.J.	mean ± S.D.
c_{max}	(ng/ml)	–	42	19	57	8	22	19	24 ± 20
t_{max}	(min.)	–	180	20	180	15	30	15	73 ± 83
F_{rel}	(mg)[1]	0	6.0	0.8	1.7	0.1	0.4	2.0	1.6 ± 2.1
F_{rel}	(% of dose)	0	3.1	0.4	0.9	0.1	0.2	1.0	0.8 ± 1.1

[1] = expressed as mg of thiazinamium methylsulphate.

The PEG suppositories were generally well tolerated. Only two of the patients reported any notable local irritation (G.L. and H.H., who both reported an unbearable irritation after application of the WH-15 suppositories!). This low degree of irritation is possibly due to the slow release of the drug from the suppositories - due to slow dissolution of the PEG - which prevents high local concentrations.

When one compares these results with those of KERCKHOFFS and HUIZINGA (1967) it appeared that our findings are quite different from those obtained by the latter authors (see, however, Part II, Chapter 2.).

5.3.3. "Ion pair absorption" studies

The results of this pilot-study are given in Table 5. For WH-15 it indicates that the reference suppositories containing thiazinamium methylsulphate gave the highest drug concentration in 24-hours' urine, namely a mean value of 5.7 ± 3.9 (S.D.) mg, which equals 0.0139 ± 0.0095 (S.D.) mmol or 2.9 ± 2.0 (S.D.)% of the dose. Five of the patients in the study described in Section 5.3.1 collected their urine for 24 hours. In these cases 2.5 ± 1.7 (S.D.)% of the dose was found in urine, which correlates well with the result of this screening test.
The ion pair thiazinamium salicylate was almost equally well absorbed as the methylsulphate, but the ion pair thiazinamium iodide was less well absorbed from WH-15. Addition of an excess of counter ions resulted in a lower degree of absorption.
Absorption from the PEG suppositories was in general much poorer than from the WH-15.
The overall results suggest that the ion pair concept of increasing the lipophilicity of the thiazinamium compound does not yield a higher degree of absorption. An explanation for these results could be that the ion pairs precipitate in the small volume of rectal fluid; the decrease in absorption after adding an excess of counter ions could support this assumption. On the other hand it may be that thiazinamium can only be absorbed as cation through intercellular pores, and not as ion pair through the lipophilic part of the membrane.
But this is theory, and it remains very difficult to draw valid conclusions from the results of the screening test. More fundamental information about the processes that occur during the period that the suppository is melting or dissolving and of those occurring after that, is necessary before a definitive judgement about a possible "ion pair absorption" process can be given.
In this respect the findings of KAKEMI and others (1969) are of interest. The

TABLE 5: *Amount of drug excreted in 24-hours' urine and irritation reported after administration of several suppositories containing thiazinamium (as cation or as ion pair).*

	WITEPSOL H-15			POLYETHYLENE GLYCOL 1500/4000 (1 : 2)		
Drug substance[1]	Suppository[1]	Amount in 24-hrs urine (mg)[2] mean ± S.D.	Irritation[3]	Suppository[1]	Amount in 24-hrs urine (mg)[2] mean ± S.D.	Irritation[3]
blank	A-1; C-1	0	0	B-1; D-1	0	0
ThMS	A-2; C-2	5.7 ± 3.9	5	B-2; D-2	0.7 ± 0.4	2
ThI	A-3	2.5 ± 2.2	1	B-3	0.9 ± 0.3	2
ThSal	C-3	4.7 ± 3.5	3	D-3	0.4 ± 0.3	1
ThMS/KI	A-4	0.3 ± 0.5	5	B-4	0.9 ± 0.6	3
ThI/KI	A-5	0.3 ± 0.5	4	B-5	0.7 ± 0.6	3
ThMS/NaSal	C-4	2.1 ± 1.2	2	D-4	1.6 ± 0.6	1
ThSal/NaSal	C-5	2.0 ± 2.1	2	D-5	1.4 ± 0.9	2

[1] = For abbreviation and code see Table 2.
[2] = Expressed as mg of thiazinamium methylsulphate.
[3] = Irritation scaled from 0-5.

authors studied absorption of ion pairs of the quaternary ammonium ions isopropamide and propantheline with laurylsulphate and saccharinate anions in rat by means of an *"in situ"* rectal perfusion technique. For the laurylsulphate ion pair the authors found no substantial increase of the percentage "absorbed" (= disappeared from the perfusion fluid). A slight increase of absorption of propantheline in the presence of saccharinate was reported (from 6.5% to 13.6%). The authors concluded that increase of the lipoid-solubility by ion pair formation alone does not explain the mechanism of a possibly increased absorption.
Local irritation was reported by several subjects, especially after application of WH-15 suppositories containing the methylsulphate. It is interesting to see that the volunteers more frequently reported irritation than the patients. This may be due to the fact that the patients remained in bed for one hour, which may result in a more pronounced spread of the molten suppository, whereas the volunteers stood up after 20 min. Moreover, psychological factors (the patients being better motivated and willing to cooperate) can play a role. Of the other suppositories those which contain potassium iodide seemed to cause strong irritation, whereas in a number of cases a correlation seemed to exist between the amount of thiazinamium cations found in urine and the degree of irritation.
The PEG suppositories were tolerated better. Although osmosis processes may cause irritation after application of PEG suppositories (BEVERNAGE and POLDERMAN, 1973) none of the subjects reported any irritation after applying blank suppositories of this PEG blend.

Conclusion

Thiazinamium cations, when applied in the form of the methylsulphate, can be absorbed from the human rectum. Two suppository bases were investigated, Witepsol H-15 and a polyethylene glycol mixture (1500/4000 in a ratio of 1 : 2). The best results were obtained with Witepsol H-15. The peak in the plasma concentration-time curve appeared about 60 min. after administration. After the maximum the curve declines rather rapidly, and generally drops to a zero or almost zero concentration in seven hours. The bioavailability obtained with Witepsol H-15 suppositories was about 6% of the dose, which is of the same order of magnitude as after oral administration of the drug. Interindividual variation was also similar to that obtained after oral administration. Again there are indications of a "first pass effect" occurring. After application of the drug in the polyethylene glycol base very low plasma concentrations are found and bioavailability is almost negligible.
Attempts to increase the absorption of thiazinamium by applying it as an ion pair were unsuccessful: absorption of thiazinamium iodide and

thiazinamium salicylate is even poorer or at best as good as absorption of the methylsulphate.

References

BEVERNAGE, K. B. M. and J. POLDERMAN (1973), *Pharm. Weekblad 108*, 429

BREIMER, D. D. (1974) in: "*Pharmacokinetics of Hypnotic Drugs*", Drukkerij Brakkenstein, Nijmegen, The Netherlands

BUCHER, K. (1948), *Helv. Physiol. Acta 6*, 821

FINGL, E. and D. M. WOODBURY (1975), in: "*The Pharmacological Basis of Therapeutics*", p. 7, L. S. Goodman and A. Gilman, Eds., Macmillan Publishing Co., Inc., New York, U.S.A.

"FORMULARIUM DER NEDERLANDSE APOTHEKERS", K.N.M.P., The Hague, The Netherlands

GOODMAN, L.S. and A. GILMAN (1975), "*The Pharmacological Basis of Therapeutics*", Macmillan Publishing Co., Inc., New York, U.S.A.

GREENLEAF, J. C. and J. W. HADGRAFT (1960), *Chem. Drug. 173*, 41

HENNIG, W. (1959), in: "*Über die Rectale Resorption von Medikamenten*", Juris Verlag, Zürich

HOLLINSHEAD, W. H. (1961), in: "*Anatomy for Surgeons*", Part II, P. B. Hoeber, New York

KAKEMI, K., H. SEZAKI, S. MURANISHI and Y. TSUJIMURA (1969), *Chem. Pharm. Bull. 17*, 1641

KERCKHOFFS, H. P. M. and T. HUIZINGA (1967), *Pharm. Weekblad 102*, 1183

LAMMERS, W., F. A. NELEMANS, TH. J. BOUWMAN, J. VAN NOORDWIJK and W. M. ROSINGA (1975), *Algemene Farmacotherapie*, Stafleu's Wetenschappelijke Uitgeversmaatschappij B.V., Leiden, The Netherlands

MÜNZEL, K. (1966), in: "*Der Einflusz der Formgebung auf die Wirkung eines Arzneimittels*", Sonderdruck aus: Fortschritte der Arzneimittelforschung, Vol. 10, p. 206 to 359, Birkhäuser Verlag, Basel, Stuttgart

RITSCHEL, W. A. (1973), in: "*Angewandte Biopharmazie*", Wissenschaftliche Verlagsgesellschaft, Stuttgart

ROSSUM, J. M. VAN (1976), in: "*Farmacokinetiek*", Cursus Post Academisch Onderwijs, K.N.M.P., The Hague, The Netherlands

SUNDWALL, A., K. UTHNE and J. VESSMAN (1971), *Acta Pharmacol. Toxicol. 29*, 385

WESTLAKE, W. J. (1973), in: "*Current Concepts in the Pharmaceutical Sciences - Dosage Form Design and Bioavailability*", J. Swarbrick, Ed., Lea & Febiger, Philadelphia, U.S.A.

"WITEPSOL® FÜR SUPPOSITORIEN", Dynamit Nobel A.G. / Chemische Werke Witten, Witten, G.F.R., 1967

6

Biotransformation and excretion - general remarks

Generally speaking, elimination of quaternary ammonium compounds from the blood is very fast. This is known to be the case for several of these drugs in the literature and it is also found to be the case with thiazinamium cations, all having relatively short biological half-lives (Chapters 2 and 3). Biotransformation and excretion of the various drugs which contain a quaternary ammonium group in the molecule, however, seem to vary widely.

Drugs can be excreted in urine by a passive or an active process or by a combination of both. The passive process, or *glomerular filtration* as it is called, occurs in the glomeruli where the blood flowing through the kidneys is ultrafiltrated. The normal renal blood flow is estimated at 1200 ml/min. The total glomerular filtration rate in the healthy adult is approximately 130 ml/min. Almost all the water of the glomerular filtrate is reabsorbed. Together with the water drug molecules can also be passively reabsorbed during the passage through the tubuli. The resulting urine production is about 1 ml/min. Some drugs are excreted from the capillaries surrounding the tubuli into the urine by an active process which is called (renal) *tubular secretion.* Like all active processes, tubular secretion is capacity-limited. Transport from the tubuli back into the blood stream is also possible (= active reabsorption). A comprehensive review on excretion of drugs by the kidney has been given by WEINER (1971).

High water-solubility combined with rather small molecular weight seems to favour rapid glomerular filtration of several quaternary ammonium compounds and hence excretion of unchanged drug in urine (*e.g.* HARINGTON, 1953; PFEFFER and others, 1968; ALBANUS and others, 1969; MEIJER and others, 1971; SIDELL and GROFF, 1971; SUNDWALL and others, 1971; BACK and CALVEY, 1972; RAABLAUB and FREY, 1972; VOJVODIC and MAKSIMOVIC, 1972; SUNDWALL and others, 1973). For some quaternary ammonium compounds an active transport system, tubular secretion, has also been described (GREEN and others, 1959; MCISAAC, 1969).

Besides renal elimination, biliary excretion of the unchanged drug can also occur rapidly, especially when the highly polar quaternary ammonium group is located at one end of the molecule while a nonpolar (= lipophilic) ring structure makes up the remaining moiety of the molecule. The processes of hepatic uptake and biliary excretion have been discussed in

detail by several authors and need not be repeated here (SCHANKER, 1965; SMITH, 1971; MEIJER, 1972, 1977). We shall confine ourselves to the observation that thiazinamium cations seem to comply with the above mentioned requirements for biliary excretion.

Apart from excretion via urine or bile a third route of elimination of quaternary ammonium compounds from the blood was suggested by LAUTERBACH (1975), namely secretion into the lumen of the small intestines. The author found indications that mechanisms exist which are able to transport quaternary ammonium compounds (test substance: tetraethylammonium bromide) against a concentration gradient from the blood into the intestinal lumen. Intestinal excretion was also mentioned by SUNDWALL and others (1971) and briefly and without supporting evidence by ALLGÉN and associates, 1960 (see below). It will be clear that, if such a process occurs, it also will ultimately result in faecal excretion, provided that no reabsorption takes place further on in the intestines. In this respect it is comparable with biliary excretion.

Theoretically there exist some other routes of elimination (*e.g.* secretion in the pancreatic juice, gastric secretion, salivary excretion) but detailed information obtained from fundamental investigation on these subjects is limited.

On the other hand the biotransformation of quaternary ammonium compounds (and successive urinary and/or biliary excretion of the metabolites) is not only determined by the occurrence of the quaternary ammonium group, but also by the chemical and physico-chemical properties of the remaining part of the molecule, *e.g.* the presence of an ester or hydroxyl group. In consequence, metabolism can vary widely for different quaternary ammonium compounds and general rules are hard to give.

Because thiazinamium methylsulphate belongs to the group of quaternary ammonium compounds containing a phenothiazine ring structure in the molecule, our attention has been focused primarily on the metabolism and excretion of this type of compounds. The investigations reported so far in the literature were mainly performed in animals and were done with labelled compounds. As far as we know, no data are available about metabolism and excretion of this type of compounds in man (*N.B.* Although several studies on the fate of non-quaternary phenothiazine derivatives in man are reported in the literature, for the reasons mentioned above, we will not discuss them here).

ALLGÉN and associates (1960) extensively studied the biological fate of the ^{35}S-labelled compound Secergan® (= 10-α-dimethyl-aminopropionyl-phenothiazine methylbromide) in the rat after subcutaneous and oral administration. After both routes of administration Secergan® was eliminated in the urine and the faeces. After subcutaneous injection 7 to 37% of the dose (= administered amount of radioactivity) was recovered in urine and 63 to 93% in faeces. The authors suggested three possible

excretion routes that would account for the drug present in the faeces: mainly by secretion in bile, to some extent by passage through the intestinal wall and probably by secretion in the pancreatic juice. After oral administration excretion in urine did not exceed 5% of the dose and elimination in the faeces accounted for 90 to 93% of the dose. The radioactive material in urine and faeces was shown by paper chromatography to be mainly unchanged Secergan®, and only traces, if any, of metabolites.

HANSSON and SCHMITTERLÖW (1961) have investigated the biotransformation and excretion of the ^{35}S-labelled quaternary phenothiazine compound Aprobit® (= 1-(10-phenothiazinylmethyl)ethyl-2-hydroxyethyldimethylammonium chloride). This compound differs only in one respect from thiazinamium methylsulphate: one of the groups attached to the nitrogen atom in the side chain is now a hydroxyethyl group instead of a methyl group. Aprobit® was administered intramuscularly and orally to rats. After intramuscular injection 57 to 64% of the dose was recovered in the urine and 17 to 18% in the faeces. After oral administration 11 to 20% was excreted in the urine and 72 to 76% of the dose was found in the faeces. In a separate study 12 to 36% of a dose injected intravenously was found to be excreted in the bile within four hours. Paper chromatographic analysis of both urine and bile samples obtained after intravenous and intramuscular injection showed four spots: unchanged Aprobit® (77 to 91%), the sulphoxide (4 to 14%) and two unidentified metabolites (3 to 11% and 1 to 2% respectively). It should be noted that in the above mentioned experiments with oral administration the authors, when dealing with the "elimination in faeces", did not take into account that the high amounts of radioactivity in the faeces were probably mainly due to unabsorbed drug substance.

HUANG and others (1970a) have studied the distribution, excretion and metabolism of the ^{14}C-labelled quaternary ammonium salt of mepazine and promethazine (as iodides) in rats (*N.B.* The authors do not indicate that the promethazine methiodide used by them is identical to thiazinamium iodide). The drugs were given by intraperitoneal injection of a suspension in sesame oil. For ^{14}C-promethazine methiodide faecal excretion was found to be the major route of elimination (59% of the dose) whereas 12% of the dose was found in the urine and 29% was unaccounted for. In a metabolic study, urine samples were investigated by paper chromatographic analysis. No other compounds except unchanged drug could be found after an injection of ^{14}C-promethazine methiodide.

Obviously the results of HUANG and others with the methiodide of promethazine are in fair contrast to the findings of HANSSON and SCHMITTERLÖW with the hydroxyethyl chloride salt of the same drug, both with regard to the biotransformation and the way of excretion. This discrepancy between results was ascribed by HUANG to the different quaternary ammonium derivatives and species used in these studies (*N.B.* In a separate

study on thiazinamium iodide in rat we found biliary excretion to be the predominant excretion process after intravenous injection, whereas a substantial amount of sulphoxide was also found; NEEF and others, 1977). The results obtained by HUANG and his associates with the ^{14}C-labelled quaternary ammonium salt of mepazine are almost identical to those reported for the promethazine derivative.
On the other hand the group of HUANG (HUANG and others, 1970c) found that the quaternary derivative of perphenazine was mainly excreted by the faecal route (40%) and only 14% was found in urine.
In a third study (HUANG and others, 1970b) this group investigated the quaternary analogues of promazine, chlorpromazine and triflupromazine. Over half (51 to 55%) the administered radioactivity was recovered in the faeces, and urinary excretion represented 8 to 30% of the administered radioactivity. Promazine methiodide and triflupromazine methiodide were excreted unchanged, but chlorpromazine methiodide was metabolized to its sulphoxide. The methyl groups attached to the terminal nitrogen atom were stable and not demethylated in the metabolic process.
It is difficult to evaluate the impact of these studies of HUANG and coworkers because a large portion of administered radioactivity was not retrieved.
In conclusion it can be stated that all the investigators cited above found both biliary and urinary excretion of unchanged drug after parenteral administration of quaternary phenothiazine derivatives. The relative amount excreted in the faeces and the urine varied for the different compounds. For all drugs biotransformation, if any, was found to be limited. If biotransformation *did* occur, the sulphoxide was always found (although only a few procent of the dose). In a few instances it was accompanied by small amounts of unidentified metabolites. For thiazinamium iodide (= promethazine methiodide) no biotransformation in rat was reported. About 59% of the dose was found to be excreted by the faecal route and 12% was detected in the urine after intraperitoneal administration.

In the present study our interests were threefold. Firstly, we investigated the biotransformation and excretion in a qualitative way after intramuscular and oral administration, in order to identify the metabolites (Chapter 7). Special attention was paid to a possible demethylation process because, if it occurred, it would lead to the formation of promethazine. This compound also has antihistaminic and anticholinergic properties (see Part I, Section 6.2) but in contrast to thiazinamium cations, promethazine molecules can penetrate into the central nervous system and produce sedation.
Secondly, we investigated the rate and extent of urinary excretion of the unchanged drug and its sulphoxide after intravenous, intramuscular, oral

and rectal administration, in order to see if the route of administration influences this process (Chapters 8, 9, 10 and 11). Biliary excretion was studied only after intramuscular injection.
Although we could not obtain information from our human experiments about the process of intestinal excretion for thiazinamium cations, this will be dealt with in the discussion of our autoradiographic studies in mice and in a monkey (see Chapter 13).
Finally we did some preliminary studies on the process of salivary excretion of thiazinamium cations, although this route of elimination has not yet been reported in literature for quaternary ammonium compounds.
The results of these studies will be dealt with in the same sequence as was used for the pharmacokinetic and bioavailability studies.

References

ALBANUS, L., A. SUNDWALL and B. VANGBO (1969), *Acta Pharmacol. Toxicol. 27,* 97

ALLGÉN, L. G., L. EKMAN, L. REIO and S. ULLBERG (1960), *Arch. Int. Pharmacodyn. 126,* 1

BACK, D. J. and T. N. CALVEY (1972), *Brit. J. Pharmacol. 46,* 355

GREEN, R. E., W. E. RICKER, W. T. ALTWOOD, Y. S. KOH and L. PETERS (1959), *J. Pharmacol. Exp. Ther. 126,* 195

HANSSON, E. and C. G. SCHMITTERLÖW (1961), *Arch. Int. Pharmacodyn. 131,* 309

HARINGTON, M. (1953), *Clin. Sci. 12,* 185

HUANG, C. L., J. A. YEH and S. Y. HSU (1970a), *J. Pharm. Sci. 59,* 772

HUANG, C. L., J. Z. YEH and I. A. MUNI (1970b), *J. Pharm. Sci. 59,* 1114

HUANG, C. L., G. M. MIR and J. Z. YEH (1970c), *J. Pharm. Sci. 59,* 976

LAUTERBACH, F. (1975), *Arzneimittel-Forsch. 25,* 479

MCISAAC, R. J. (1969), *J. Pharmacol. Exp. Ther. 168,* 6

MEIJER, D. K. F. (1972), in: *"On Hepatic Uptake and Biliary Excretion of Quaternary Ammonium Compounds"*, thesis, State University, Groningen

MEIJER, D. K. F. (1977), in: *"Intestinal Permeation"* (M. Kramer and F. Lauterbach, Eds.), p. 196, Excerpta Medica, Amsterdam

MEIJER, D. K. F., G. A. VERMEER and G. KWANT (1971), *Europ. J. Pharmacol. 14,* 280

NEEF, C., J. H. G. JONKMAN and D. K. F. MEIJER (1977), in: *"Abstracts of Symposia and Communications of the 18th Dutch Federative Meeting"*

PFEFFER, M., J. M. SCHOR, S. BOLTON and R. JACOBSEN (1968), *J. Pharm. Sci. 57,* 1375

RAABLAUB, J. and P. FREY (1972), *Arzneimittel-Forsch. 22,* 73

SCHANKER, L. S. (1965), in: *"The Biliary System"* (W. Taylor, Ed.), Blackwell Scientific Publications, Oxford

SIDELL, F. R. and W. A. GROFF (1971), *J. Pharm. Sci. 60,* 1224

SMITH, R. L. (1971), in: *"Concepts in Biochemical Pharmacology"*, Part 1, p. 354 (B. B. Brodie and J. R. Gilette, Eds.), Springer Verlag, Berlin, New York

SUNDWALL, A., K. UTHNE and J. VESSMAN (1971), *Acta Pharmacol. Toxicol. 29,* 385

SUNDWALL, A., J. VESSMAN and B. STRINDBERG (1973), *Europ. J. Clin. Pharmacol. 6,* 191

VOJVODIC, V. B. and M. MAKSIMOVIC (1972), *Europ. J. Clin. Pharmacol. 5,* 58

WEINER, I. M. (1971), in: *"Concepts in Biochemical Pharmacology"*, Part 1, p. 328 (B. B. Brodie and J. R. Gilette, Eds.), Springer Verlag, Berlin, New York

7

Biotransformation and excretion in man: a qualitative study

Contents

7.1. Introduction

Urine samples from volunteers and patients were examined after oral and intramuscular administration of thiazinamium methylsulphate in order to obtain an impression of the biotransformation processes. Special attention was paid to two processes. Firstly, we investigated to see if any sulphoxidation occurred, being the most common biotransformation reaction for phenothiazine derivatives in general (CIACCIO, 1971; HIRTZ, 1971; LA DU and others, 1971; WILLIAMS, 1971; SAUNDERS, 1974).
Secondly, we investigated to see if thiazinamium cations were demethylated to the tertiary amine promethazine. Demethylation is a common biotransformation process for tertiary amines. Although no evidence exists in the literature about the possibility of demethylation of quaternary ammonium compounds in man, we were interested in this process because the resulting product, promethazine, is of therapeutic interest (see Chapter 6). Because phenothiazine derivatives containing a tertiary amine group are easily oxidized in the body, we decided to screen the urine both for promethazine and its sulphoxide.
A general screening test for biotransformation products in urine – collected after all the previously described routes of administration – was performed using thin-layer chromatography. This method would probably also detect possible aromatic hydroxylation products of thiazinamium, hydroxylation also being a principal metabolic pathway for phenothiazine derivatives.

Apart from the urine samples also some bile samples obtained after intramuscular injection could be examined in this way (see Section 9.2.2.3.). Additional analyses were performed by gas chromatography and by radiochemical techniques.

7.2. Materials and methods

Gas chromatographic analysis

Urine samples (5 ml) were extracted at pH = 10 with n-hexane and analyzed by means of gas chromatography according to the method described for phenothiazines in general by CURRY and MOULD (1969) (see Part II, Section 2.1.).

Thin-layer chromatographic analysis

System I: Urine samples (20 ml) were evaporated by a process of freeze-drying. Methanol (500 µl) was added to the residue. After thoroughly mixing 20 µl of the supernatant was applied to a thin-layer plate. Development was performed in methanol (100%) and subsequently in the same direction in a mixture of water (20 ml), methanol (100 ml) and ammonium acetate (3 g). An U.V. detection method was used. The technical details and descriptions of the reagents used were given in Part II, Chapter 3.

System II: Isolation from urine and bile samples (up to 20 ml) was performed by means of Amberlite XAD-2® column chromatography and a separation was carried out by means of two-dimensional thin-layer chromatography as described in Part II, Chapter 3.

Reference substances

Promethazine hydrochloride and thiazinamium methylsulphate were obtained from SPECIA, Rhône Poulenc, Paris, France. Promethazine sulphoxide hydrochloride and thiazinamium sulphoxide methylsulphate were kindly supplied by the same company.

Promethazine sulphoxide hydrochloride and thiazinamium sulphoxide iodide were prepared in our own laboratory as described in Part II, Chapter 3. (*N.B.* No difference was found in thin-layer chromatographic behaviour between the methylsulphate and the iodide of thiazinamium).

Radiochemical analysis

Radiochemical analyses were performed as described in Section 9.2.2.3.
In order to control whether any unchanged drug and/or possible metabolite(s) were lost in the aqueous eluates of the column or were not eluated by methanol due to irreversible binding to the Amberlite XAD-2® material, the following control experiments were performed. One ml of all aqueous eluates was mixed with 10 ml of Aquasol®, scintillation liquid, and the radioactivity was estimated. Portions of approximately 1 gram of the remaining Amberlite XAD-2®, after elution had been performed, were also submitted to the counting procedure.
These control experiments were performed with all the urine and bile samples obtained in the study described in Section 9.2.2.3.

7.3. Results and discussion

In a preliminary *thin-layer chromatographic analysis* urine samples obtained at various intervals after oral and intramuscular administration of thiazinamium methylsulphate were examined according to System I. The development in methanol was carried out in order to remove several interfering compounds from the urine or bile sample and to obtain a separation between possibly occurring tertiary amines (promethazine and its sulphoxide) and quaternary ammonium compounds (thiazinamium cation and its sulphoxide). The latter compounds stayed at the spotting place during this development. After the second development a good separation was obtained between the four compounds mentioned.
This system was improved later resulting in System II.
With both methods, after analyzing the urine samples, only two spots could be detected which proved to be thiazinamium cations and its sulphoxide. No evidence was obtained for the presence of promethazine and promethazine sulphoxide.
Also in the bile and urine samples of the four patients, who received an

intramuscular injection of 12.5 mg, only thiazinamium cations and thiazinamium sulphoxide cations could be detected (see also Chapter 9). Urine samples obtained at various intervals after oral and intramuscular injection were also screened by a *gas chromatographic method* which was preceded by an extraction method which enables the isolation of non-quaternary phenothiazine derivatives. In this way promethazine and promethazine sulphoxide could be extracted from the urine, but thiazinamium could not, neither its sulphoxide. In none of these samples was any promethazine or promethazine sulphoxide detectable.
The method of *radiochemical analysis* was used for the urine and bile samples of a patient who had received an intramuscular injection of ^{35}S-labelled thiazinamium methylsulphate (see Chapter 9). Isolation was performed by means of column chromatography with Amberlite XAD-2® as described in Part II, Chapter 3. The aqueous eluates, used for washing the column, and the remaining Amberlite XAD-2® material did not contain any radioactivity after finishing the total procedure. This means that all radioactivity submitted to the procedure was now present in the methanol eluate. This final methanol solution was concentrated as described in Part II, Chapter 3 and submitted to the two-dimensional thin-layer chromatographic method of System II. Again only two spots were found, which were identified as thiazinamium and thiazinamium sulphoxide, both in the urine and in bile.
The quantitative chemical analysis of the two spots and the determination of the total radioactivity in the samples correlated well with each other. The differences found were within the 95% confidence limits of the methods. This finding suggests that little or no other ^{35}S-containing metabolites, *e.g.* ring-hydroxylation products, were present.

Conclusion

The results mentioned above suggests that sulphoxidation is the major process of biotransformation for thiazinamium cations after parenteral, oral and rectal administration in humans.
Attempts to detect the demethylation product were unsuccessful, suggesting that this process does not take place.
Also no indications of ring-hydroxylation could be found.

References

CIACCIO, E. I. (1971), in: *"Drill's Pharmacology in Medicine"* (J. R. Dipalma, Ed.) 4th ed., Mc. Graw-Hill Book Company, New York
CURRY, S. S. and G. P. MOULD (1969), *J. Pharm. Pharmacol. 21*, 674

HIRTZ, J. L. (1971), *"Analytical Metabolic Chemistry of Drugs"*, p. 53, Marcel Dekker, Inc., New York

LA DU, B. N., H. G. MANDEL and E. L. WAY (1971), *"Fundamentals in Drug Metabolism and Drug Disposition"*, The William & Wilkins Company, Baltimore

SAUNDERS, L. (1974), *"The Absorption and Distribution of Drugs"*, Baillière Tindall, London

WILLIAMS, R. T. (1971), in: *"Concepts in Biochemical Pharmacology"*, Part 2, (B. B. Brodie and J. R. Gilette, Eds.), Springer Verlag, Berlin, New York

8

Biotransformation and excretion after intravenous injection in man

Contents

8.1. Introduction

After finding that the administration of thiazinamium methylsulphate results in the excretion in the urine of unchanged thiazinamium cations and thiazinamium sulphoxide cations (see Chapter 7), we decided to study this phenomenon in a quantitative way after an intravenous injection.

8.2. Materials and methods

Subjects and conditions

The patients described in Section 2.2 participated in this study. All the circumstances, materials and methods are described there. Urine was collected every hour, if possible, until one hour after the last blood sample was taken.

Dosage form

See Part III, Section 2.2.

Determination of thiazinamium cations in urine

See Part II, Chapter 2.

Determination of thiazinamium sulphoxide cations in urine

See Part II, Chapter 3.

8.3. Results and discussion

Fig. 1 shows the renal excretion rate and the cumulative renal excretion of thiazinamium cations and thiazinamium sulphoxide cations in patient J. C. The total amounts excreted of both the unchanged drug and the metabolite are given for all seven patients in Table 1.
The urinary excretion of thiazinamium cations was fast especially during the first hours after injection. No correlation could be found with the rate of urine production. The relatively large urinary excretion rate suggests that the highly water-soluble thiazinamium cations can easily leave the bloodstream.
In cases of linear kinetics the rate of excretion of a drug in the urine is given by the following equation:

$$\frac{dQ_r}{dt} = Cl_r \cdot c_p \qquad \text{(XVI)}$$

where: $\frac{dQ_r}{dt}$ = renal excretion rate (the amount of drug eliminated in a certain period by renal excretion) (*e.g.* ng/min.)
Cl_r = renal clearance; that part of the total body clearance ($= Cl_{tot}$) that occurs by renal excretion (ml/min.)
c_p = plasma concentration (ng/ml)

So, the renal excretion rate as function of time is a direct reflection of the profile of the plasma concentration-time curve.
A plot of the renal excretion rate (the amount of drug excreted in a certain time period) *versus* the average plasma concentration over the excretion interval can serve as a tool to investigate whether, apart from the process of glomerular filtration, any non-linear excretion mechanism plays a role, such as tubular secretion. When glomerular filtration is the only process of

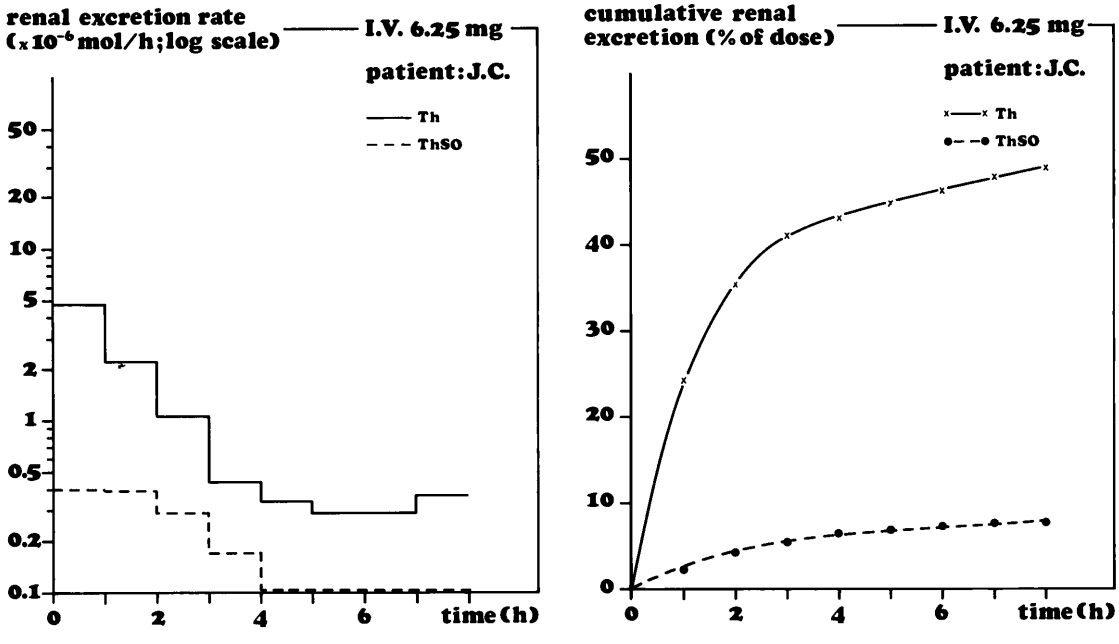


FIG. 1: *Typical examples of renal excretion rate curves (left, on semi-logarithmic scale) and cumulative renal excretion curves (right) of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intravenous injection of a dose of 6.25 mg. Patient: J.C.*

renal excretion, then the plot will be linear. If not, the curve shows a deflection due to the fact that the tubular secretion process is limited by a certain fixed maximum transport value (RENNICK and others, 1954; VAN GINNEKEN, 1976). However, construction of such a plot from the urinary excretion data of thiazinamium cations obtained after intravenous injection of a dose of 6.25 mg is difficult due to the fact that from the plasma concentration data it became apparent that we are dealing with two-compartment kinetics with extremely fast distribution during the first two hours ($(t_{\frac{1}{2}})_\alpha = \pm$ 6 min.), followed by a slower decay ($(t_{\frac{1}{2}})_\beta$ is about 2 hours). Reliable results can be obtained by this method only when the urine collection periods are short when compared to the biological half-life, so that a large number of urinary excretion data are obtained over a wide range of plasma concentrations. Unfortunately this prerequisite was not fulfilled in our experiments, but we found a rough indication that the curve may not be linear and that tubular secretion may be involved. Although we were aware of the limited degree of precision of this method, due to the same reasons already mentioned, we have also estimated the values of the renal clearance by applying equation XVI and have found them to vary from 107

TABLE I: *Cumulative urinary excretion and half-lives of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intravenous injection of a dose of 6.25 mg (= 19.8 × 10^{-6} mol). Half-lives obtained from urinary excretion deficit curves.*

Patient		J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	mean ± S.D.
Amount excreted:									
Th	(× 10^{-6} mol)	9.8	9.9	9.6	7.3	6.0	4.4	10.2	8.2 ± 2.3
Th	(% of dose)	49.6	50.1	48.7	37.1	30.3	22.3	51.5	41.4 ± 11.6
ThSO	(× 10^{-6} mol)	1.5	2.4	2.8	1.7	1.1	1.6	1.7	1.8 ± 0.6
ThSO	(% of dose)	7.8	12.1	14.2	8.6	5.4	7.9	8.6	9.2 ± 2.9
Th + ThSO	(× 10^{-6} mol)	11.3	12.3	12.4	9.0	7.1	6.0	11.9	10.0 ± 2.6
Th + ThSO	(% of dose)	57.4	62.2	62.9	45.7	35.7	30.2	60.1	50.5 ± 13.3
Th : ThSO		1 : 0.15	1 : 0.24	1 : 0.29	1 : 0.23	1 : 0.18	1 : 0.36	1 : 0.17	1 : 0.22
Half-lives:									
Th	(min.)	118	112	120	126	110	94	98	111 ± 12
ThSO	(min.)	110	120	78	96	82	86	110	97 ± 16

to 663 ml/min. which corresponded with 28 to 65% (mean 48 ± 14 (S.D.)) of the total plasma clearance in the same patient. The values of the total plasma clearance are given in Chapter 2, Table 1 (mean 606.9 ± 459.8 ml/min.). All values estimated for the renal clearance were substantially larger than glomerular filtration as measured by creatinine clearance (130 ml/min.), which would form another indication of an active excretion process.

Although we will discuss this phenomenon in more detail in the following chapters, it is worthwhile mentioning that for some other quaternary ammonium compounds tubular secretion was also reported. RENNICK and others (1954) found tubular secretion for tetraethylammonium ions in dogs and chickens. SWARTZ and SIDELL (1974) reported renal tubular secretion for pralidoxime chloride in man. A renal clearance of 612 ± 9 ml/min. was reported.

For the determination of the half-life of elimination, we used the method of the renal deficit (VAN ROSSUM, 1971; BREIMER, 1974), also named the ARE method (Amount of drug Remaining to be Excreted; RITSCHEL, 1976). This method can only be used when urine has been collected until essentially all unchanged drug has been excreted (the cumulative urinary excretion curve runs parallel to the abscissa). For single compartment kinetics the following equation can be used for the calculation of the biological half-life.

$$\log (Q_{r,\,t=\infty} - Q_{r,\,t}) = \log Q_{r,\,t} - 0.301 \cdot t/t_{\frac{1}{2}} \qquad \text{(XVII)}$$

where:

$Q_{r,\,t=\infty}$ = the total amount of drug excreted by renal excretion

$Q_{r,\,t}$ = the amount excreted by renal excretion until time t

$(Q_{r,\,t=\infty} - Q_{r,\,t})$ = the excretion deficit (amount remaining to be excreted)

So, for a one-compartment open model the log of the renal excretion deficit is a linear function of time.

We found in most patients that after an intravenous dose of 6.25 mg the urinary excretion was complete or almost complete at the end of the experiment. The cumulative excreted amount varied from 22.3 to 51.5% of the dose with a mean value of 41.4 ± 11.6 (S.D.)%. These results allow us to use the method of renal deficit. Concerning the other prerequisite (single compartment kinetics) the following can be observed. Pharmacokinetic analysis of the plasma concentration data (Chapter 2) has led us to assume that a two-compartment open model is valid for thiazinamium cations. But as was shown in Fig. 1 in Chapter 2 the influence of the distribution process on the profile of the plasma concentration-time curve was already limited after about 120 min. Then, elimination becomes the predominant process during the next part of the curve. For that reason, the corresponding part of the plasma concentration-time curve is linear on semilogarithmic scale. If, for the sake of simplicity, only this part of the curve is used, it can be considered as a one-compartment model. This is reflected in the renal excretion deficit *versus* time curve: the log of the renal excretion deficit is a linear function of time in the latter part of the curve (see Fig. 2). As a consequence the elimination half-life can be calculated from these curves. The values obtained by graphical analysis for the seven patients are given in Table 1. There appeared to exist only a slight variation in the elimination half-lives of thiazinamium cations in the seven patients: a mean value of 111 ± 12 (S.D.) was found.

Biotransformation to the sulphoxide only seemed to occur to a rather low extent: 5.4 to 14.2% of the dose (mean value 9.2 ± 2.9 (S.D.)%) was found as sulphoxide in urine. The urinary excretion of thiazinamium sulphoxide cations was very rapid and was generally complete at the end of the experiment (t = 480 min.). The elimination half-lives obtained from urinary excretion deficit curves appeared to be almost identical to those found for thiazinamium cations: a mean value of 97 ± 16 (S.D.) min. was calculated. This implies that the rate of sulphoxidation is probably the rate limiting step for the elimination of the sulphoxide.

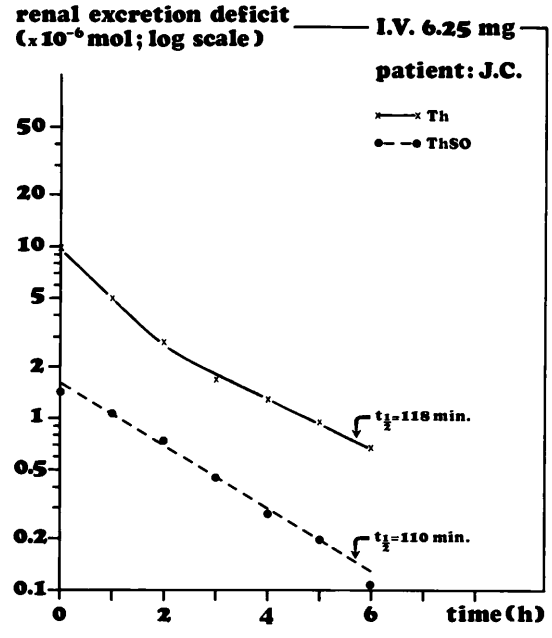


FIG. 2: *Renal excretion deficit curves on semi-logarithmic scale of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intravenous injection of a dose of 6.25 mg. Patient : J.C. The data have been obtained from the values of the cumulative renal excretion.*

The total urinary excretion of thiazinamium cations and thiazinamium sulphoxide cations - calculated on a molecular base - seemed to account for about half of the dose: mean value 50.5 ± 13.3 (S.D.)% of the dose. The average molecular ratio between unchanged drug and metabolite was 1 : 0.22.

Comparison of these results with the findings of other investigators is hardly possible because reports on this subject are scarce. As was discussed in Chapter 6, HUANG and others (1970) have studied the fate of thiazinamium iodide after intraperitoneal injection in rats. These authors found only 12% of the dose in urine as unchanged drug. Contrary to our study in man, HUANG and his associates could not find any biotransformation products in the urine of the rats.

Conclusion

After intravenous injection of thiazinamium methylsulphate about 41.4% of the dose was excreted unchanged in the urine. Excretion was very rapid

and almost complete within 8 hours. About 9.2% of the dose was excreted in the urine in the form of thiazinamium sulphoxide cations.
The mean value for the ratio between the amounts of unchanged drug and sulphoxide in urine was 1 : 0.22.
Indications were found that active secretion processes in the tubuli play an important role in the elimination of thiazinamium cations in the urine.

References

BREIMER, D. D. (1974), in: *"Pharmacokinetics of Hypnotic Drugs"*, thesis, Drukkerij Brakkenstein, Nijmegen, The Netherlands

GINNEKEN, C. A. M. VAN (1976), in: *"Pharmacokinetics of Antipyretic and Anti-inflammatory Analgetics"*, thesis, University of Nijmegen, The Netherlands

HUANG, C. L., J. A. YEH and S. Y. HSU (1970), *J.Pharm. Sci. 59,* 772

RENNICK, B. R., D. M. CALHOON, H. GANDIA and G. K. MOE (1974), *J. Pharmacol. Exp. Ther. 110,* 309

RITSCHEL, W. A. (1976), in: *"Handbook of Basic Pharmacokinetics"*, Drug Intelligence Publication, Hamilton, Illinois 62341, U.S.A.

ROSSUM, J. M. VAN (1971), in: *"Drug Design"*, Vol. 1, Chapter 7 (E. J. Ariëns, Ed.), Academic Press, New York

SWARTZ, R. D. and F. R. SIDELL (1974), *Proc. Soc. Exp. Biol. Med. 146,* 419

9

Biotransformation and excretion after intramuscular injection in man

Contents

9.1. Introduction

The fate of thiazinamium cations in man after an intramuscular injection was investigated. Special attention was paid to the excretion rate of the unchanged drug and the metabolite: thiazinamium sulphoxide cations.
A number of studies were performed with different doses and different aims. They will be discussed in the same sequence that we have used before in the discussion on the pharmacokinetics after intramuscular injection (Chapter 3).

With the low dose a comparison with the intravenous administration was made.
Also two intramuscular doses were compared.
In some bile-fistula patients both urinary and biliary excretion were subject to investigation.

9.2. Materials and methods

9.2.1. Investigations with a dose of 6.25 mg

Patients, circumstances, materials and methods were identical to those described in Sections 2.2, 3.2.1 and 8.2.

9.2.2. Investigations with a dose of 12.5 mg

9.2.2.1. Comparison with an intramuscular dose of 25.0 mg

Nine male patients participated in this study. Seven of them have already been described in Section 3.2.2.1. The two other patients (J.S. and R.W.), aged 23 and 22, weighed 86 and 83 kg respectively.
The circumstances, materials and methods were identical to those described in Sections 2.2, 3.2.2.1 and 8.2.

9.2.2.2. Comparison with rectal administration

The patients, conditions, materials and methods were identical to those described in Sections 2.2, 3.2.2.2 and 8.2, except that in most instances the urine was collected over a period of 24 hours.

9.2.2.3. Investigations involving bile-fistula patients

Subjects and conditions

These investigations were performed in the hospital "Diaconessenhuis" at Eindhoven and were supervised by DR. J. BENDER, M.D. and by DRS. A. M. SOETERBOEK, clinical pharmacist.
Four patients were participating in this study and all had been given complete details concerning the nature of the experiment and consented to cooperate. They were not suffering from generalized obstructive lung diseases, but were hospitalized because of *cholelithiasis*. During the operation (*cholecystectomy* with *choledochotomy*) a T-drain was fixed in the *ductus choledochus*, so that bile could be collected afterwards. Patient characteristics were given in Table 4. The results of the conventional tests on the liver function of these patients were normal. The experiment started two days after the operation. An intramuscular injection was given as described in Sections 3.2.1 and 3.2.2. Urine and bile were collected at different times as given in Table 4. The patients did not receive a special diet. Comedication could not be avoided and is also given in Table 4.
All other conditions, materials and methods were the same as described in Sections 2.2, 3.2.2.1 and 8.2.
One patient (A.A.) consented to receive an intramuscular injection of the ^{35}S-labelled compound.

Dosage form

For patients G.S., K.S. and S.H. an aqueous solution containing thiazinamium methylsulphate, as described in Section 2.2, was used. Patient A.A. received an injection of 11.57 mg ^{35}S-labelled thiazinamium methylsulphate. The substance was prepared at The Radiochemical Centre, Amersham, England, and was kindly supplied by SPECIA, Rhône Poulenc, Paris, France. Specific activity during the experiment was 6.0 μCi/mg, so in total the patient received a dose of 69.4 μCi. The radiation burden was calculated by DRS. A. VERSLUIS, Department of Nuclear Medicine, University Hospital, Groningen (Director: PROF. DR. M. G. WOLDRING) and was found to be well within the limits.

Determination of thiazinamium cations

For plasma samples the method described in Part II, Section 2.3 was used; for urine that described in Part II, Section 2.4. Bile samples were analyzed according to method A described in Part II, Section 2.5.

Determination of thiazinamium sulphoxide cations

For urine and bile analysis the methods described in Part II, Sections 3.3 and 3.4 were used.

Radiochemical analysis

All radiochemical analyses were performed in the Department of Nuclear Medicine, University Hospital, Groningen (Director: PROF. DR. M. G. WOLDRING). The total activity in all urine and bile samples was determined as follows: 500 µl fractions of the samples were discoloured by standing overnight with 5 drops of hydrogen peroxide (30%) and 1 drop of 4 n NaOH at 37° C. To the residue 10 ml of a scintillation medium was added (Aquasol®, New England Nuclear, Boston, U.S.A.) and the radioactivity was estimated with a liquid scintillation spectrometer (Nuclear Chicago Mark II).
Fractions of the urine and bile samples were also submitted to the same method of analysis as described in Part II, Chapter 3 (column chromatography with Amberlite XAD-2®, followed by two-dimensional thin-layer chromatography). A total scan of the plates was made (qualitative measurement) and the amount of radioactivity of the thiazinamium and thiazinamium sulphoxide spots was determined by means of an L.B. 2723 Berthold Dünnschicht Scanner II.

9.2.3. Investigations with a dose of 25.0 mg

9.2.3.1. Comparison with an intramuscular dose of 12.5 mg

The patients participating in this study were the same as described in Section 9.2.2.1.
All other conditions, materials and methods were the same as those described in Sections 2.2, 3.2.2.1 and 8.2.

9.2.3.2. Comparison with oral administration

Subjects and conditions

The ten patients participating in this study were already described in Section 3.2.3.2.

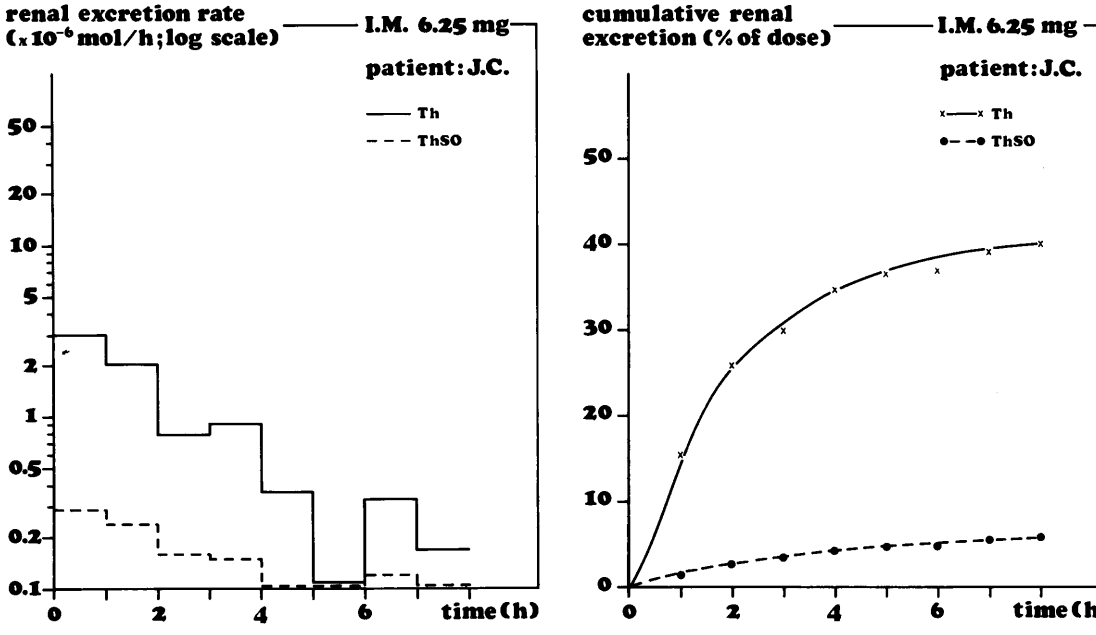


FIG. 1: *Typical examples of renal excretion rate curves (left, on semi-logarithmic scale) and cumulative renal excretion curves (right) of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intramuscular injection of a dose of 6.25 mg. Patient: J.C.*

Details on four other patients (D.V., W.B., J.S. and R.W.) are given in Table 5 in Chapter 10.
All other circumstances, materials and methods have been described in Sections 2.2, 3.2.3.2 and 4.2.1.

9.3. Results and discussion

9.3.1. Investigations with a dose of 6.25 mg

Fig. 1 shows the renal excretion rate and the cumulative renal excretion of thiazinamium cations and thiazinamium sulphoxide cations in patient J.C. Table 1 summarizes the excretion details of the unchanged drug and the metabolite for all seven patients.

TABLE I: *Cumulative urinary excretion and half-lives of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intramuscular injection of a dose of 6.25 mg (= 19.8 × 10^{-6} mol). Half-lives obtained from urinary excretion deficit curves.*

Patient		J.C.	U.D.	J.B.	H.L.	H.G.	H.B.	H.J.L.	mean ± S.D.
Amount excreted:									
Th	(× 10^{-6} mol)	7.9	9.6	7.5	7.6	6.0	5.5	8.7	7.5 ± 1.4
Th	(% of dose)	39.8	48.7	38.2	38.6	30.3	27.8	43.9	38.2 ± 7.3
ThSO	(× 10^{-6} mol)	1.1	1.8	1.4	1.6	1.0	1.0	1.0	1.3 ± 0.3
ThSO	(% of dose)	5.4	8.9	7.3	8.1	5.1	5.1	5.1	6.4 ± 1.6
Th + ThSO	(× 10^{-6} mol)	9.0	11.4	8.9	9.2	7.0	6.5	9.7	8.8 ± 1.6
Th + ThSO	(% of dose)	45.2	57.6	45.4	46.7	35.4	32.9	49.0	44.6 ± 8.3
Th : ThSO		1 : 0.14	1 : 0.19	1 : 0.19	1 : 0.21	1 : 0.17	1 : 0.18	1 : 0.11	1 : 0.16
Half-lives:									
Th	(min.)	98	92	98	94	106	84	102	96 ± 7
ThSO	(min.)	124	82	74	102	104	90	156	105 ± 28

The excretion of thiazinamium cations appeared to be rapid. The maximum in the plasma concentration-time curve was found to occur after about 6 min., followed by a very fast decay. So, it is not surprising that, especially in the first hours, a considerable amount of the drug was excreted. For most of the patients urinary elimination was complete or almost complete at the end of the experiments after 8 hours. The total amount of excreted drug - in the unchanged form - varied from 27.8 to 48.7% of the dose, with a mean value of 38.2 ± 7.3(S.D.)%. These observations are quite similar to those obtained after an intravenous injection in the same patients. In both cases the lowest amount was excreted by patient H.B., although excretion was found to be complete. Patient U.D. had excreted the highest amount in both cases. No significant difference could be found between the mean values obtained for the total amount excreted after intravenous and after intramuscular administration (Student's t-test; $p > 0.05$).

The elimination half-life of thiazinamium cations was 96 ± 7(S.D.) min. The observed values in general show a good similarity to those found after intravenous administration.

Just as was found after intravenous administration, biotransformation to the sulphoxide occurred only to a low extent after intramuscular injection: 5.1 to 8.9% of the dose (mean value of 6.4 ± 1.6(S.D.)%) was excreted in

the urine in the form of the sulphoxide. Again no significant difference could be found ($p > 0.05$). For the half-life of elimination of the sulphoxide cations a mean value of 105 ± 28(S.D.) min. was found.
The sum of the urinary excretion of thiazinamium cations and thiazinamium sulphoxide cations was 44.6 ± 8.3 (S.D.)% of the dose, which is not significantly different from the value found after intravenous administration ($p > 0.05$).
The average molecular ratio between drug and metabolite was 1 : 0.16.

9.3.2. Investigations with a dose of 12.5 mg

9.3.2.1. Comparison with an intramuscular dose of 25.0 mg

The results of this study are presented in Table 5. Fig. 3a shows examples of the renal excretion rate and the cumulative renal excretion of thiazinamium cations and thiazinamium sulphoxide cations. The observations will be discussed in detail in Section 9.3.3.1 where also a comparison will be made with the results obtained after giving a dose of 25.0 mg to the same patients.
The 12.5 mg study was undertaken with nine patients. The results led us to the conclusion that two of them had to be considered separately, namely J.S. and R.W., because of an extremely low degree of sulphoxidation. This phenomenon will be discussed in more detail in the next chapter in Section 10.3.1.
Although the aim of this study was primarily a comparison with a dose of 25.0 mg in the same patients, one is also tempted to look back to the results of the 6.25 mg study involving a different group of patients (Table 1). When comparing the mean percentage values and ratios of Table 1 and Table 5 a reasonable degree of similarity is seen, indicating that the conclusions drawn in Section 9.3.1 are also valid here.

9.3.2.2. Comparison with rectal administration

The results of this study are represented in Table 2. Two patients (H.H. and W.B.) collected their urine during 7 hours, the other five patients also collected urine over the period from 7 to 24 hours after administration (*N.B.* In the other investigations urine was collected up to 8 hours after administration).
Again, the excretion of the thiazinamium cations was very rapid. About 90% of the amount excreted during the 24 hours was found in the samples collected during the first 7 hours. During the total period of 24 hours 31.9

TABLE 2: *Cumulative urinary excretion of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intramuscular injection of a dose of 12.5 mg (= 39.6 × 10^{-6} mol).*

Patient		G.L.	P.H.	W.M.	H.H.[1]	W.B.[1]	M.F.	T.J.	mean ± S.D.[2]
Th	(× 10^{-6} mol)	17.6	12.0	13.9	15.3	13.6	11.7	8.0	12.6 ± 3.5
Th	(% of dose)	44.4	30.3	35.1	38.6	34.3	29.6	20.2	31.9 ± 8.8
ThSO	(× 10^{-6} mol)	2.9	3.0	4.1	0.8	2.3	3.1	2.7	3.2 ± 0.6
ThSO	(% of dose)	7.3	7.6	10.4	2.0	5.8	7.8	6.8	8.0 ± 1.4
Th + ThSO	(× 10^{-6} mol)	20.5	15.0	18.0	16.1	15.9	14.8	10.7	15.8 ± 3.7
Th + ThSO	(% of dose)	51.7	37.9	45.5	40.6	40.1	37.4	27.0	39.9 ± 9.3
Th : ThSO		1 : 0.16	1 : 0.25	1 : 0.29	1 : 0.05	1 : 0.17	1 : 0.26	1 : 0.34	1 : 0.25

[1] = urine collected up to 7 h after administration
[2] = mean of 5 patients (H.H and W.B. omitted)

± 8.8(S.D.)% of the dose was excreted in the form of unchanged drug. Although the collection period was longer, this quantity was somewhat smaller than the excreted amount in a study with the same dose involving another group of patients (see Table 5). The difference may be due to interindividual variation in excretion pattern.
The same applies to the excretion of the sulphoxide. In this study 8.0 ± 1.4(S.D.)% of the dose was excreted as sulphoxide.
In total, 39.9 ± 9.3(S.D.)% of the dose was excreted in the urine, which is almost identical to that found in the other studies with the intramuscular administration.
The average ratio between the total amount of thiazinamium cations and thiazinamium sulphoxide cations excreted during the course of the study was 1 : 0.25.

9.3.2.3. Investigations involving bile-fistula patients

Fig. 2 shows the cumulative biliary and the cumulative renal excretion of thiazinamium cations and thiazinamium sulphoxide cations in patient A.A. Table 3 represents the total excreted amount of unchanged drug and the metabolite in urine and in bile in all four patients. In Table 4 the patient characteristics, dose, collection time and the amount of urine and bile collected are given.
The data of the urinary excretion are similar to those described in the previous sections: 39.9 ± 2.2(S.D.)% of the dose was found in the urine in the form of unchanged drug, and 10.6 ± 1.5(S.D.)% of the dose in the form

TABLE 3: *Cumulative urinary and biliary excretion and the sum of both of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intramuscular injection in bile-fistula patients. Patient characteristics, dose etc. are given in Table 4.*

Patient		G.S.			K.S.			S.H.			A.A.			mean ± S.D.	mean ± S.D.	mean ± S.D.
		urine	bile	total	urine	bile	total	urine	bile	total	urine	bile	total	urine	bile	total
Th	($\times 10^{-6}$ mol)	17.0	5.9	22.9	15.8	8.7	24.5	14.9	5.0	19.9	11.0	4.7	15.7			
Th	(% of dose)	42.9	14.9	57.8	39.9	22.0	61.9	37.6	12.6	50.2	39.0	16.7	55.7	39.9 ± 2.2	16.6 ± 4.0	56.4 ± 4.9
ThSO	($\times 10^{-6}$ mol)	4.1	3.6	7.7	4.1	3.7	7.8	5.0	2.7	7.7	2.5	0.7	3.2			
ThSO	(% of dose)	10.4	9.1	19.5	10.4	9.3	19.7	12.6	6.8	19.4	8.9	2.5	11.4	10.6 ± 1.5	6.9 ± 3.2	17.5 ± 4.1
Th + ThSO	($\times 10^{-6}$ mol)	21.1	9.5	30.6	19.9	12.4	32.3	19.9	7.7	27.6	13.5	5.4	18.9			
Th + ThSO	(% of dose)	53.3	24.0	77.3	50.3	31.3	81.6	50.3	19.4	69.7	47.9	19.1	67.0	50.5 ± 2.2	23.5 ± 5.7	73.9 ± 6.7
Th : ThSO		1 : 0.24	1 : 0.64	1 : 0.34	1 : 0.26	1 : 0.43	1 : 0.32	1 : 0.34	1 : 0.54	1 : 0.39	1 : 0.23	1 : 0.15	1 : 0.20	1 : 0.27	1 : 0.42	1 : 0.31

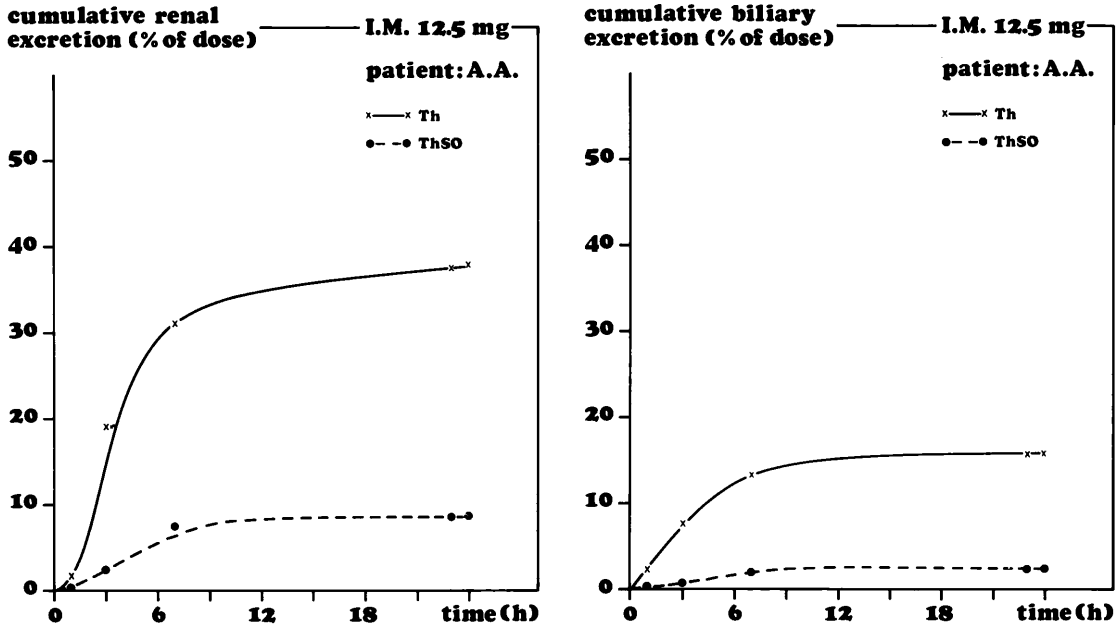


FIG. 2: *Cumulative excretion curves of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) in urine (left) and bile (right) after intramuscular injection of a dose of - about - 12.5 mg. Patient: A.A.*

of the sulphoxide. In total about half of the dose was found to be excreted in the urine: 50.5 ± 2.2(S.D.)% of the dose. It is interesting to note from the results involving patient A.A. that the urinary excretion within a period of 24 hours after administration was completed: the sample collected between the 23rd and 24th hour did not contain any detectable amount of thiazinamium or sulphoxide cations.

As compared to the previous experiments, the ratio between the unchanged drug and metabolite is comparable, namely 1 : 0.27. This finding suggests that the extent of sulphoxidation was not influenced by the comedication.

Substantial amounts of both thiazinamium cations and thiazinamium sulphoxide cations were also found in the bile samples (see Fig. 2). The cumulative amount of unchanged drug in bile varied from 12.6 to 22.0% of the dose, with a mean value of 16.6 ± 4.0(S.D.)% of the dose. The excretion appeared to be complete within the 24 hours of the experiment because no drug could be detected in samples collected between the 23rd and 24th hour following drug administration. Although the total amount of bile collected varied from 105 to 450 ml, no correlation could be found between the amount of bile excreted and the amount of drug it contained.

The discovery that thiazinamium cations can easily be excreted into the bile is not surprising. It completely fulfils the requirements for biliary excretion as postulated by SCHANKER (1965) by having a polar quaternary ammonium group and a lipophilic ring structure in the molecule.

TABLE 4: *Patient characteristics, dose, urine and bile production, collection times, and comedication of the bile-fistula patients participating in the study described in Section 9.3.2.3.*

Patient		G.S.	K.S.	S.H.	A.A.
Sex		female	male	female	female
Age	(year)	66	68	58	55
Body weight	(kg)	65	68	60	58
Height	(m)	1.70	1.85	1.68	1.65
Dose*	(mg)	16.22	16.22	16.22	11.57
	(mg/kg)	0.2495	0.2385	0.2703	0.1995
	($\times 10^{-6}$ mol)	39.6	39.6	39.6	28.2
	($\times 10^{-6}$ mol/kg)	0.609	0.582	0.660	0.486
24 hrs urine	(ml)	1250	1550	650	1210
collecting time	(h)	6,24	7,24	7,24	1,3,7,23,24
24 hrs bile	(ml)	450	264	180	105
collecting time	(h)	24	7,24	1,3,7,23,24	1,3,7,23,24
Comedication		Sintrom® Dulcolax® Mogadon®	Penbritin® Euphyllin® vit.B complex	digoxin Dolviran® Sintrom® Baralgin® Mogadon®	Vibramycin® pilocarpine heparin

Also a substantial amount of thiazinamium sulphoxide cations were found in bile. In two patients the cumulative amounts of sulphoxide cations in bile were almost identical to that in the urine. In the two others lower amounts were found. The cumulative excreted amount of the metabolite in bile varied from 2.5 to 9.3% of the dose with a mean value of 6.9 ± 3.2(S.D.)%. Again, excretion appeared to be complete within 24 hours. Excretion of such considerable amounts of the sulphoxide in bile is rather surprising because the introduction of an oxygen atom in the molecule markedly decreases the lipophilic character of the phenothiazine group. This can be concluded from the chromatographic behaviour and the dissolution properties of the sulphoxide as compared to those of the parent drug (see Part II, Chapter 3). The latter data would suggest that the sulphoxide does not fulfil the prerequisites for biliary excretion as well as thiazinamium itself. But apparently the physico-chemical properties of the sulphoxide are such that biliary excretion *can* occur.

Furthermore, it is interesting to note that the average ratio between the unchanged drug and metabolite was 1 : 0.42, whereas this ratio in urine was found to be considerably lower, namely 1 : 0.27.

Discussing the results obtained by biliary excretion in bile-fistula patients it is important to note that only an unknown part of the total bile

production can be collected by means of a T-drain. Of course, the situation of the patients during this study is different from conditions which would occur naturally. As a consequence there may be slight differences between our findings and the extent these processes occur in a normal situation. For example, enterohepatic cycling - if it occurs at all - is almost impossible under the conditions of the experiment. However, this is the best approximation.

The total amounts of drug and metabolite recovered from urine plus bile varied from 67.0 to 81.6% of the dose with a mean value of 73.9 ± 6.7(S.D.)% of the dose. This value consists of an average total value of thiazinamium cations of 56.4 ± 4.9(S.D.)% of the dose and an average amount of thiazinamium sulphoxide cations of 17.5 ± 4.1(S.D.)% of the dose. The remaining, unrecovered, part of the dose may reflect the amount of bile that was not collected, assuming that the sulphoxide is the only metabolite of thiazinamium and that the urinary and biliary excretion are the only routes of elimination. After correction for unrecovered bile we found for the amount of thiazinamium cations excreted in bile a mean value of 35.5 ± 7.2(S.D.)% of the dose and for the sulphoxide 14.2 ± 5.1(S.D.)% of the dose. This led us to the conclusion that thiazinamium cations are excreted in urine and in bile in almost identical proportions, whereas for the sulphoxide the biliary route prevails.

9.3.3. Investigations with a dose of 25.0 mg

9.3.3.1. Comparison with an intramuscular dose of 12.5 mg

Results obtained with a dose of 12.5 mg are represented in Table 5 and those obtained in the same patients with a dose of 25.0 mg are given in Table 6.

TABLE 5: *Cumulative urinary excretion of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intramuscular injection of a dose of 12.5 mg (= 39.6 × 10⁻⁶ mol).*

Patient		A.O.	K.W.	A.V.	E.D.	N.S.	E.W.	E.P.	mean ± S.D.	J.S.	R.W.
Th	(× 10^{-6} mol)	12.1	15.3	15.3	16.1	13.2	14.1	14.3	14.3 ± 1.4	10.0	13.6
Th	(% of dose)	30.7	38.6	38.6	40.7	33.3	35.7	36.0	36.2 ± 3.4	25.2	34.3
ThSO	(× 10^{-6} mol)	3.0	1.1	3.1	0.8	2.1	2.3	0.8	1.9 ± 1.0	0.2	0.3
ThSO	(% of dose)	7.5	2.8	7.8	1.9	5.4	5.8	2.0	4.7 ± 2.5	0.5	0.8
Th + ThSO	(× 10^{-6} mol)	15.1	16.4	18.4	16.9	15.3	16.4	15.1	16.2 ± 1.2	10.2	13.9
Th + ThSO	(% of dose)	38.2	41.4	46.4	42.6	38.7	41.5	38.1	40.9 ± 3.1	25.8	35.1
Th : ThSO		1 : 0.25	1 : 0.07	1 : 0.20	1 : 0.05	1 : 0.16	1 : 0.16	1 : 0.06	1 : 0.13	1 : 0.02	1 : 0.02

TABLE 6: *Cumulative urinary excretion of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intramuscular injection of a dose of 25.0 mg (= 79.1 × 10^{-6} mol).*

Patient	A.O.	K.W.	A.V.	E.D.	N.S.	E.W.	E.P.	mean ± S.D.	J.S.	R.W.
Th (× 10^{-6} mol)	8.0	42.5	22.5	26.5	24.4	20.5	19.7	23.4 ± 10.3	29.0	21.0
Th (% of dose)	10.1	53.7	28.5	33.5	30.9	25.9	24.9	29.6 ± 13.0	36.7	26.6
ThSO (× 10^{-6} mol)	2.3	2.6	6.4	2.0	3.2	3.9	1.2	3.1 ± 1.7	1.3	0.6
ThSO (% of dose)	2.9	3.3	8.1	2.6	4.1	4.9	1.5	3.9 ± 2.1	1.7	0.8
Th + ThSO (× 10^{-6} mol)	10.3	45.1	28.9	28.5	27.6	24.4	20.9	26.5 ± 10.5	30.3	21.6
Th + ThSO (% of dose)	13.0	57.0	36.6	36.1	34.9	30.8	26.4	33.5 ± 13.2	38.4	27.4
Th : ThSO	1 : 0.29	1 : 0.06	1 : 0.28	1 : 0.08	1 : 0.13	1 : 0.19	1 : 0.06	1 : 0.13	1 : 0.04	1 : 0.03

In Fig. 3b a typical example is given of the renal excretion rate and the cumulative renal excretion of thiazinamium and thiazinamium sulphoxide cations.

After receiving a dose of 12.5 mg, in most patients the renal excretion is essentially complete after 8 hours, but after the 25.0 mg dose it was not. In the case of the lower dose 36.2 ± 3.4(S.D.)% of the dose was found in the urine in the form of the unchanged drug, whereas after the 25.0 mg dose a mean value of 29.6 ± 13.0(S.D.)% of the dose was found.

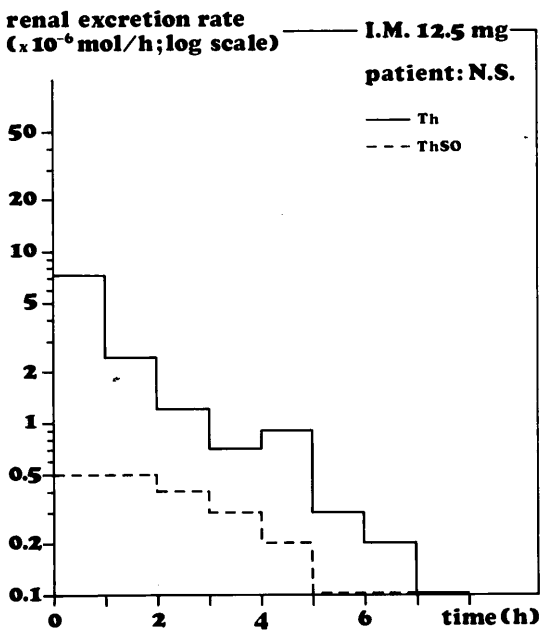
In this study the pH of every urine sample was measured. No correlation could be found between the pH of the urine sample, varying between physiological limits, and the amount excreted in it. This is understandable if one realizes that both compounds are completely ionized at all pH values. So, no unionized form exists and processes like passive tubular reabsorption must be considered as very unlikely.

Excretion of the sulphoxide was also slightly lower after the 25.0 mg dose: 4.7 ± 2.5(S.D.)% of the dose was excreted as sulphoxide after an injection of 12.5 mg and 3.9 ± 2.1(S.D.)% after the 25.0 mg dose.

The total excretion after the 12.5 mg dose is 40.9 ± 3.1(S.D.)%, whereas it accounts for a value of 33.5 ± 13.2(S.D.)% of the dose after the 25.0 mg dose.

It is notable that the ratio between the quantity of unchanged drug and metabolite in urine was found to be almost identical in each individual after application of the two doses.

Special attention should be paid to two of the patients (J.S. and R.W.) where hardly any sulphoxide was found in urine, namely about 1% of the dose or less, resulting in a ratio between unchanged drug and metabolite of 1 : 0.02 in both subjects after the 12.5 mg, and 1 : 0.04 and 1 : 0.03 after the 25.0 mg dose. We will discuss this phenomenon in detail in Section 10.3.1.



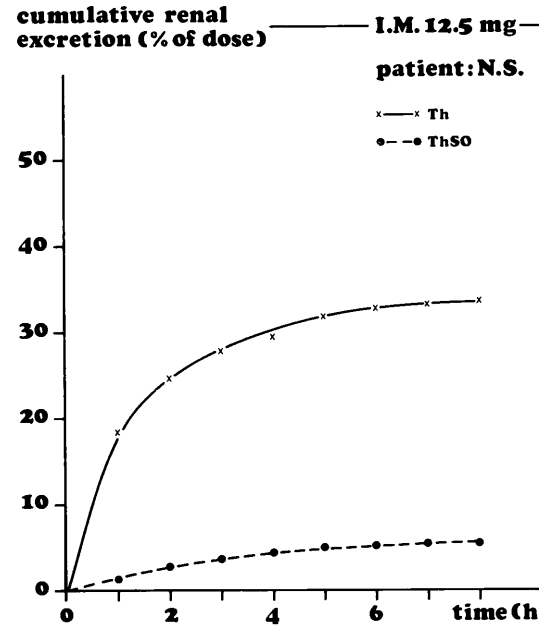
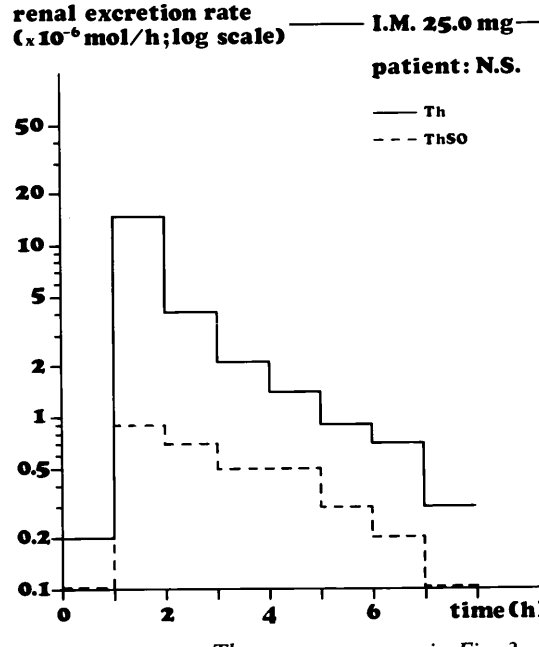


FIG. 3a: *Typical examples of renal excretion rate curves (left, on semi-logarithmic scale) and cumulative renal excretion curves (right) of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intramuscular injection of a dose of 12.5 mg. Patient: N.S.*



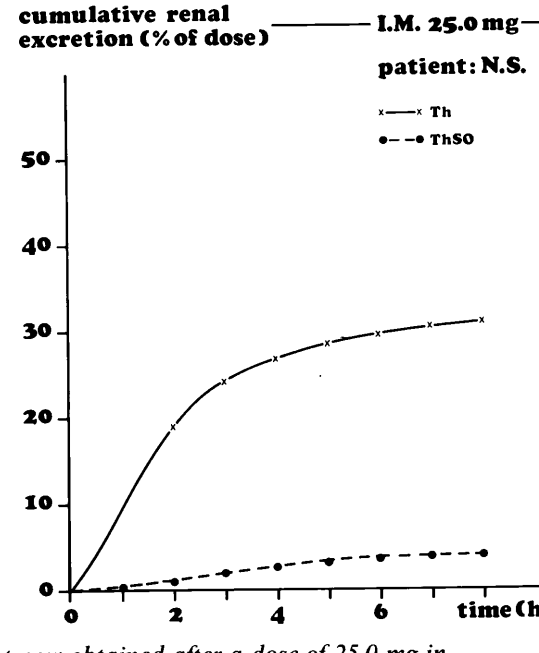


FIG. 3b: *The same curves as in Fig. 3a, but now obtained after a dose of 25.0 mg in the same patient.*

TABLE 7: *Cumulative urinary excretion of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after intramuscular injection of a dose of 25.0 mg (= 79.1 × 10^{-6} mol).*

Patient		F.K.	J.A.M.	D.M.	D.H.	O.V.	S.W.	mean ± S.D.[1]	mean ± S.D.[2]	D.V.	W.B.	J.S.	R.W.	mean ± S.D.
Th	(× 10^{-6} mol)	17.1	12.5	10.3	12.6	25.8	22.6	15.3 ± 8.7	20.0 ± 10.3	28.8	27.1	29.0	21.0	26.5 ± 3.8
Th	(% of dose)	21.6	15.8	13.0	15.9	32.6	28.6	21.3 ± 7.9	25.3 ± 13.0	36.5	34.4	36.7	26.6	33.6 ± 4.7
ThSO	(× 10^{-6} mol)	2.8	5.0	2.8	2.0	5.9	2.7	3.5 ± 1.5	3.5 ± 1.7	0.6	1.2	1.3	0.6	0.9 ± 0.4
ThSO	(% of dose)	3.7	6.3	3.7	2.5	7.5	3.5	4.5 ± 1.9	4.5 ± 2.1	0.8	1.6	1.7	0.8	1.2 ± 0.5
Th + ThSO	(× 10^{-6} mol)	19.9	17.5	13.1	14.6	31.7	25.3	20.4 ± 7.0	23.5 ± 10.6	29.4	28.3	30.3	21.6	27.4 ± 4.0
Th + ThSO	(% of dose)	25.3	22.1	16.7	18.4	40.1	32.1	25.8 ± 8.9	29.8 ± 13.3	37.3	36.0	38.4	27.4	34.8 ± 5.0
Th : ThSO		1 : 0.16	1 : 0.40	1 : 0.27	1 : 0.16	1 : 0.23	1 : 0.12	1 : 0.23	1 : 0.18	1 : 0.02	1 : 0.04	1 : 0.04	1 : 0.03	1 : 0.03

[1] = mean value of the six patients listed in this table

[2] = mean value of all ten patients

9.3.3.2. Comparison with oral administration

Excretion data of six of the patients participating in this study are given in Table 7. The data of the four other participating patients (A.O., K.W., A.V. and E.D.) have been represented already in Table 6. In Table 7 the mean values are given for the group of six patients as well as for the group of ten patients. The results are similar to those already described in Section 9.3.3.1 and Table 6.
Again, a low ratio between unchanged drug and metabolite was seen in patients J.S. and R.W. In two other patients (D.V. and W.B.) this phenomenon was also observed. We will discuss it in more detail in Section 10.3.1.

Conclusion

After intramuscular injection of thiazinamium methylsulphate the urinary excretion of unchanged cations is rapid. After a dose of 6.25 mg or 12.5 mg the excretion is essentially complete within 8 hours, but this is not the case after the administration of a dose of 25.0 mg. No correlation could be found between urine production or pH and the amount of drug excreted in urine. About 30 to 40% of the dose was excreted as thiazinamium cations and 5 to 10% of the dose was excreted as thiazinamium sulphoxide cations. The ratio between the unchanged drug and the metabolite was found to be a rather fixed value in each patient, but substantial interindividual variations were seen. The mean value for this ratio was about 1 : 0.20. Some patients formed almost no sulphoxide (ratio about 1 : 0.03).
In a study involving bile-fistula patients it was found that both thiazinamium cations and thiazinamium sulphoxide cations are excreted to a considerable extent in bile. After a correction for the assumed loss of bile, it was found that almost identical amounts of unchanged drug were excreted in the urine and bile, whereas the sulphoxide was excreted to a slightly higher degree in bile. The ratio between unchanged drug and metabolite in bile was about 1 : 0.42.

Reference

SCHANKER, L. S. (1965), in: *"The Biliary System"* (W. Taylor, Ed.), Blackwell Scientific Publications, Oxford

10

Biotransformation and excretion after oral administration in man

Contents

10.1. Introduction

The fate of thiazinamium cations after oral application in man was investigated with two doses. Special attention was given to the relative amount of sulphoxidation occurring, and a comparison was made with the amount of sulphoxidation occurring after an intramuscular administration of the drug.

10.2. Materials and methods

10.2.1. Investigations with a dose of 300 mg

Subjects and conditions

The ten patients, already described in Section 4.2.1, together with the four patients (D.V., W.B., J.S. and R.W.) mentioned in Section 9.2.3.2. participated in this study.
All other conditions, materials and methods were the same as described in Section 2.2, 3.2.3.2. and 4.2.1.

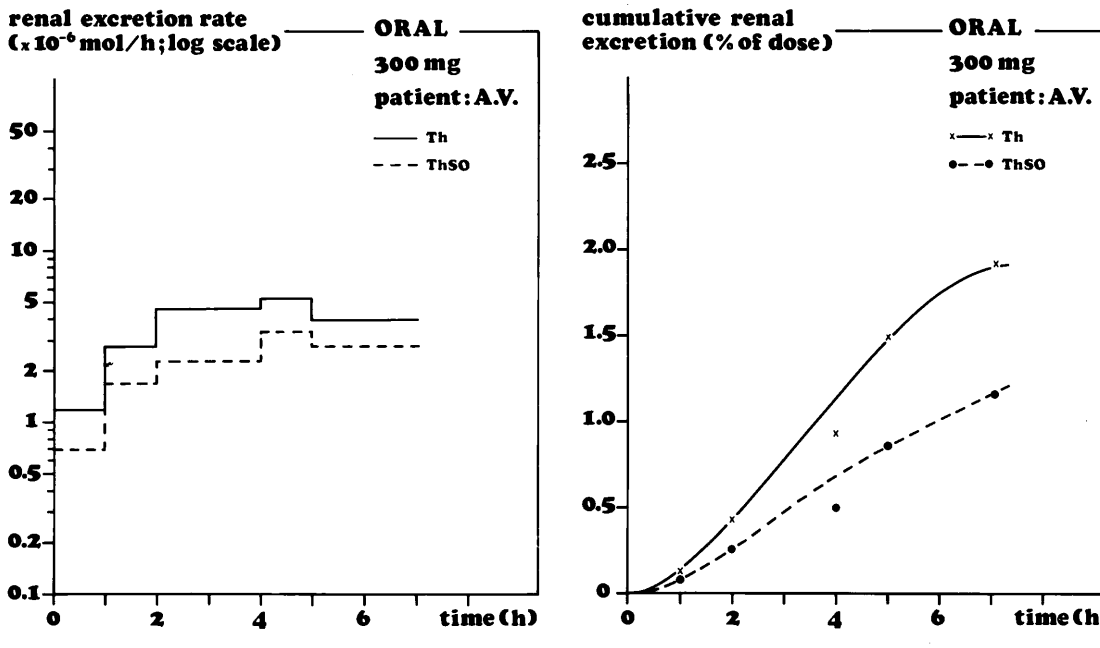


FIG. 1: *Typical examples of renal excretion rate curves (left, on semi-logarithmic scale) and cumulative renal excretion curves (right) of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after oral administration of a dose of 300 mg. Patient: A.V.*

10.2.2. Investigations with a dose of 900 mg

The patients, conditions, materials and methods were identical to those described in the previous section.

10.3. Results and discussion

10.3.1. Investigations with a dose of 300 mg

Fig. 1 shows the renal excretion rate and the cumulative renal excretion of thiazinamium cations and thiazinamium sulphoxide cations in patient A.V. Table 1 presents the amounts of these compounds excreted during the time of the experiment by all fourteen patients.

TABLE 1: *Cumulative urinary excretion of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after oral administration of a dose of 300 mg (= 94.9 × 10^{-6} mol).*

Patient		F.K.	J.A.M.	D.M.	D.H.	A.O.	O.V.	S.W.	K.W.	A.V.	E.D.	mean ± S.D.	D.V.	W.B.	J.S.	R.W.	mean ± S.D.
Th	(× 10^{-6} mol)	6.3	2.8	18.0	1.2	9.2	7.3	3.3	21.2	18.1	15.1	10.3 ± 7.3	11.0	19.2	37.9	29.0	24.3 ± 11.7
Th	(% of dose)	0.7	0.3	1.9	0.1	1.0	0.8	0.3	2.2	1.9	1.6	1.1 ± 0.8	1.2	2.0	4.0	3.1	2.6 ± 1.2
Th	(% of F_{rel})	25.6	16.4	30.1	7.4	20.7	27.4	19.3	65.7	40.5	27.4	28.1 ± 15.9	25.1	40.5	32.7	51.9	37.6 ± 11.4
ThSO	(× 10^{-6} mol)	10.2	10.3	23.8	2.9	9.5	17.9	6.0	3.7	11.0	2.1	9.7 ± 6.8	0.7	0.7	1.9	1.6	1.2 ± 0.6
ThSO	(% of dose)	1.2	1.2	2.7	0.3	1.1	2.0	0.7	0.4	1.2	0.2	1.1 ± 0.8	< 0.1	< 0.1	0.2	0.2	0.2 ± 0.1
ThSO	(% of F_{rel})	41.3	60.2	39.8	18.0	21.3	66.5	35.1	11.5	24.7	3.8	32.2 ± 20.3	1.6	1.5	1.6	2.9	1.9 ± 0.7
Th + ThSO	(× 10^{-6} mol)	16.5	13.1	41.8	4.1	18.7	25.2	9.3	24.9	29.1	17.2	20.0 ± 10.8	11.7	19.9	39.8	30.6	25.5 ± 12.3
Th + ThSO	(% of dose)	1.9	1.5	4.6	0.4	2.1	2.8	1.0	2.6	3.1	1.8	2.1 ± 1.1	1.2	2.0	4.2	3.3	2.7 ± 1.3
Th + ThSO	(% of F_{rel})	66.9	76.6	69.9	25.4	42.0	93.9	54.4	77.2	65.2	31.2	60.3 ± 21.8	26.7	42.0	34.3	54.8	39.5 ± 12.0
Th : ThSO		1 : 1.62	1 : 3.68	1 : 1.32	1 : 2.42	1 : 1.03	1 : 2.45	1 : 1.82	1 : 0.17	1 : 0.61	1 : 0.14	1 : 0.94	1 : 0.06	1 : 0.04	1 : 0.05	1 : 0.06	1 : 0.05

TABLE 2: *Cumulative urinary excretion of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after oral administration of a dose of 900 mg (= 284.8 × 10^{-6} mol).*

Patient		F.K.	J.A.M.	D.M.	D.H.	A.O.	O.V.	S.W.	K.W.	A.V.	E.D.	mean ± S.D.	D.V.	W.B.	J.S.	R.W.	mean ± S.D.
Th	(× 10^{-6} mol)	44.3	8.4	20.1	4.5	19.0	17.9	8.8	27.6	22.4	7.3	18.0 ± 11.9	59.5	62.3	65.0	23.7	52.6 ± 19.4
Th	(% of dose)	1.6	0.3	0.7	0.2	0.7	0.6	0.3	1.0	0.8	0.2	0.6 ± 0.4	2.1	2.2	2.3	0.8	1.9 ± 0.7
Th	(% of F_{rel})	29.4	8.0	5.3	6.1	11.3	19.6	31.0	32.3	21.9	8.3	17.3 ± 10.8	21.3	21.2	28.5	20.3	22.8 ± 3.8
ThSO	(× 10^{-6} mol)	40.2	18.3	11.7	7.0	15.7	24.8	22.1	7.6	20.8	1.9	17.0 ± 11.0	2.8	4.0	4.6	1.2	3.2 ± 1.5
ThSO	(% of dose)	1.4	0.6	0.4	0.2	0.6	0.9	0.8	0.3	0.7	0.1	0.6 ± 0.4	0.1	0.1	0.2	< 0.1	0.1 ± 0.1
ThSO	(% of F_{rel})	26.6	17.4	3.1	9.4	9.3	27.2	77.5	8.9	20.3	2.2	20.2 ± 22.0	1.0	1.4	2.0	1.0	1.4 ± 0.5
Th + ThSO	(× 10^{-6} mol)	84.5	26.7	31.8	11.5	34.7	42.7	30.9	35.2	43.2	9.2	35.0 ± 20.8	62.3	66.3	69.6	24.9	55.1 ± 20.2
Th + ThSO	(% of dose)	3.0	0.9	1.1	0.4	1.3	1.5	1.1	1.3	1.5	0.3	1.2 ± 0.7	2.2	2.3	2.4	0.9	2.0 ± 0.7
Th + ThSO	(% of F_{rel})	56.0	25.4	8.4	15.5	20.7	46.8	108.5	41.2	42.2	10.5	37.5 ± 29.8	23.5	23.5	30.5	21.3	24.7 ± 4.0
Th: ThSO		1 : 0.91	1 : 2.18	1 : 0.58	1 : 1.56	1 : 0.83	1 : 1.39	1 : 2.51	1 : 0.28	1 : 0.93	1 : 0.26	1 : 0.94	1 : 0.05	1 : 0.06	1 : 0.07	1 : 0.05	1 : 0.06

As was pointed out in Section 4.3.1. in only four patients, namely F.K., J.A.M., D.M. and S.W. had the plasma concentrations fallen to zero or extrapolation to zero concentration was possible within the time of the experiment. So, the bioavailability could be measured. In these patients the urinary excretion was also almost complete or could be determined by extrapolation. It was found that 22.6 ± 6.2(S.D.)% of the amount of drug which had been systemically available was excreted as unchanged drug in the urine. This value was also used to estimate the bioavailability after oral administration from the urinary excretion data in a group of nine volunteers (see Section 4.2.). In the six other patients the absorption and excretion processes were still continuing after eight hours. The total amount of thiazinamium cations excreted at the end of the experiment is 28.1 ± 15.9(S.D.)% of the amount of drug which was calculated to be systemically available during the time of the experiment (relative bioavailability). This value is comparable to the value obtained after intramuscular injection of 25.0 mg : 25.3 ± 13.0%. By application of equation XVI (Section 8.3) the renal clearance Cl_r was calculated from the results of the 300 and 900 mg experiment. Now that we are dealing with plasma concentrations over a rather wide concentration range, the calculations have increased precision. The values obtained are given in Table 3, together with the total plasma clearance values obtained by using the NAFFIT-1 programme after giving an intramuscular dose of 25.0 mg (see Section 3.3.3.2). The values for renal clearance varied from 118 to 417 ml/min. All patients except A.O. had a clearance value for thiazinamium cations which is higher than the average creatinine clearance (about 130 ml/min., RITSCHEL, 1976). This again indicates that tubular secretion occurs in the kidneys. The mean value for renal clearance was 256 ± 136(S.D.) ml/min., whereas the total plasma clearance was found to be 780 ± 450 (S.D.) ml/min. So, renal clearance is lower, in some patients even considerably lower, than the total plasma clearance. Expressed as percentage, renal clearance varied from 11 to 94% of the plasma clearance. These values suggest that we are dealing with a high hepatic clearance. In the case of thiazinamium cations in man, hepatic clearance includes the biliary excretion of the unchanged drug and the biotransformation to the sulphoxide. The values for the hepatic clearance can be calculated by subtracting the renal clearance from the total plasma clearance under the assumption that no other elimination routes exist. The hepatic clearance varied from 17 to 1515 ml/min. with a mean value of 537 ± 495(S.D.) ml/min.

With regards to the renal excretion of the metabolite, Fig. 1 shows that this process started at a lower rate than the excretion of the unchanged drug, but soon the excretion rates were almost identical. The average quantity of thiazinamium sulphoxide cations found in the urine after oral administration expressed as percentage of the bioavailability was considerably higher than after parenteral administration, namely a mean value of 32.2 ±

20.3(S.D.)% was found (see Table 1). This results in a quite different ratio between the unchanged drug and the sulphoxide in urine. The ratio had a mean value of 1 : 0.94 but appeared to show a strong interindividual variation, namely from 1 : 0.14 to 1 : 3.68. On the other hand after parenteral administration this ratio was 1 : 0.20. The total excreted amount (unchanged drug plus metabolite) was now about 60% of the amount of drug which had been available in the general circulation.

From Table 3 it becomes apparent that two correlations exist.

Firstly, patients with a high hepatic clearance of thiazinamium cations (*e.g.* J.A.M., D.H. and O.V.) have excreted a relatively large amount of the metabolite in urine. The calculated hepatic clearance in these patients is above 820 ml/min., whereas the ratio between unchanged drug and metabolite in the urine is above 1 : 2.42 in the 300 mg experiment. On the other hand, Table 3 shows that patients with a lower hepatic clearance (*e.g.* E.D., A.V. and K.W.) have excreted only a small quantity of sulphoxide in urine, with ratios between 1 : 0.14 and 1 : 0.61.

Secondly, Table 3 shows that in patients with a relatively high hepatic clearance (> 820 ml/min.) a low bioavailability was observed. When the liver plasma flow is assumed to be about 1000 ml/min., this means that approximately 80% of the drug is eliminated from the plasma by the liver in these patients. On the other hand in patients with a low hepatic clearance a better bioavailability was seen.

TABLE 3: *Total plasma clearance (Cl_{tot}), renal clearance (Cl_r) and hepatic clearance (Cl_h) of the individual patients. Th : ThSO refers to the ratio between thiazinamium cations and thiazinamium sulphoxide cations in urine. Quotations "300" and "900" refer to the dose of 300 or 900 mg respectively.*

Patient		F.K.	J.A.M.	D.M.	D.H.	A.O.	O.V.	S.W.	K.W.	A.V.	E.D.	mean ± S.D.
Cl_{tot}	(ml/min.)	892	1023	692	1337	659	1854	498	598	481	292	780 ± 450
Cl_r	(ml/min.)	401	203	392	152	118	339	260	397	417	275	256 ± 136
Cl_h	(ml/min.)	491	820	300	1182	541	1515	238	201	64	17	537 ± 495
Th : ThSO	"300"	1 : 1.62	1 : 3.68	1 : 1.32	1 : 2.42	1 : 1.03	1 : 2.45	1 : 1.82	1 : 0.17	1 : 0.61	1 : 0.14	1 : 0.94
Th : ThSO	"900"	1 : 0.91	1 : 2.18	1 : 0.58	1 : 1.56	1 : 0.83	1 : 1.39	1 : 2.51	1 : 0.28	1 : 0.93	1 : 0.26	1 : 0.94
F_{rel}	"300"	2.6	1.8	6.3	1.7	4.7	2.8	1.8	3.4	4.7	5.8	3.6 ± 1.7
Absorption[1]	"300"	6.3	7.7	12.9	5.5	8.0	9.8	4.9	3.8	6.2	6.2	7.1 ± 2.6
F_{rel}	"900"	5.3	3.7	13.4	2.6	5.9	3.2	1.0	3.0	3.6	3.1	4.5 ± 3.4
Absorption[1]	"900"	9.3	10.3	17.6	6.2	9.1	6.9	3.4	3.7	5.9	3.7	7.6 ± 4.3

[1] = Absorption was calculated by correction for "first pass effect" (see text).

The latter two findings, together with the observation that after oral administration a higher percentage of drug was excreted in the form of the metabolite, suggest that the liver plays a predominant role in bioavailability and the fate of thiazinamium cations in man. The quantitative difference in biotransformation found after oral and parenteral administration of the drug and the relation between hepatic clearance and the bioavailability values can be explained by assuming that a large amount of the drug is eliminated from the blood by the liver directly after the absorption during the first liver passage. This latter process is called "first pass effect" or "first pass metabolism" and reduces the systemic availability of the drug.
GIBALDI and PERRIER (1974) have made the following statement about "first pass effect": "Drug uptake and elimination in the liver during the first passage of drug into the circulation after hepatic route administration may effectively preclude a significant fraction of the administered dose from reaching the peripheral sampling site. This phenomenon is generally referred to as the 'first pass effect'." Where "elimination" in the liver includes biotransformation and "hepatic route administration" emphasizes oral ingestion (assuming no lymphatic absorption), intraperitoneal injection or injection in splenic or portal veins.
It is assumed that the thiazinamium cations extracted from the portal blood flow will be excreted partly as unchanged drug in the bile, whereas another part will be subjected to sulphoxidation. The sulphoxide formed in this way may be excreted in bile, whereas another part of it will reach the general circulation and will be excreted by way of the kidney and the liver. As thiazinamium sulphoxide does not have any anticholinergic or antihistaminic activity at all (ETRILLARD, *Personal Communication*, 1976) this "first pass effect" substantially reduces the - already incomplete - absorbed amount of drug, resulting in a restricted quantity of pharmacologically active substance in the general circulation.
Theoretically a higher amount of the sulphoxide in the urine can also be explained by oxidation in the acid milieu of the stomach and subsequent absorption of the sulphoxide (if possible at all).
But the correlation between the hepatic clearance and extent of biotransformation (*i.e.* "first pass effect") as mentioned above argues against it.
More evidence for the existence of a substantial "first pass effect" was obtained from animal experiments. These experiments were performed by us at the Department of Clinical Pharmacy, St. Radboud Hospital, Catholic University, Nijmegen, and were supervized by DR. E. VAN DER KLEIJN. Although we hope to publish the results of this study elsewhere, we will discuss the experiments briefly here.

Introduction: In order to avoid the oxidation in the gastrointestinal tract and to avoid irregular and incomplete absorption, the hepatic elimination of the drug during the first passage through the liver was investigated by determination of the

TABLE 4: *Data of the four dogs used in the experiment with linear infusion of thiazinamium methylsulphate in the portal vein. Absolute bioavailability (= F_{abs}) expressed as percentage of the dose.*

Dog		268	343	367	515
Breed		Labrador	Dalmatinian	Labrador	Labrador
Sex		female	male	female	male
Weight	(kg)	22	26	19	24
Dose*	(mg/kg/h)	1	1	1	1
F_{abs}	(%)	72.0	78.0	51.0	51.7

absolute bioavailability obtained after infusion of a drug containing solution in the portal vein.

Materials and methods: Dogs (details see Table 4) were anesthetized by intravenous injection of pentobarbital sodium (30 mg/kg). Anesthesia was maintained by inhalation of oxygen, nitrous oxide and fluothane. Each dog was used in two experiments with an interval of about two weeks.

In the first experiment a slow continuous infusion of thiazinamium methylsulphate in sterile physiological salt solution was given for a period of 100 min. by means of a pump (20 ml/h) in the *vena cephalica antebrachii sinister* (dose: 1 mg/kg/h). During and after the infusion blood samples (about 6 ml) were taken from the *vena saphena parva dexter* by means of a permanent cannula (Braunule®) at the time intervals indicated in Fig. 2.

In the second experiment the procedure as described was repeated but now the infusion was given in the *vena porta* (portal vein). Abdomen was opened and a cannula inserted via a side branch.

Plasma concentrations were analyzed as described in Part II, Chapter 2.3, and the absolute bioavailability was determined by the cutting and weighing of a standard type of paper.

The results of these animal experiments are summarized in Table 4. Plasma concentration curves are represented in Fig. 2. After the intravenous injection steady state plasma concentrations of about 600 ng/ml were obtained in all the dogs within the time of the experiment. After stopping the infusion plasma concentrations fell steeply. After infusion in the portal vein the plasma concentration curve slowly rises. In two dogs (343 and 367) the plasma concentration curves reached a plateau level of about 300 to 400 mg/ml. This can be explained by assuming that a substantial part of the drug offered to the liver by portal blood flow is extracted by this organ before the drug can enter the general circulation. In two other dogs (268 and 515) the curve is still going up at the end of the infusion time, suggesting that the liver systems became saturated and were no longer able to remove the drug from the portal vein to the same extent as before.

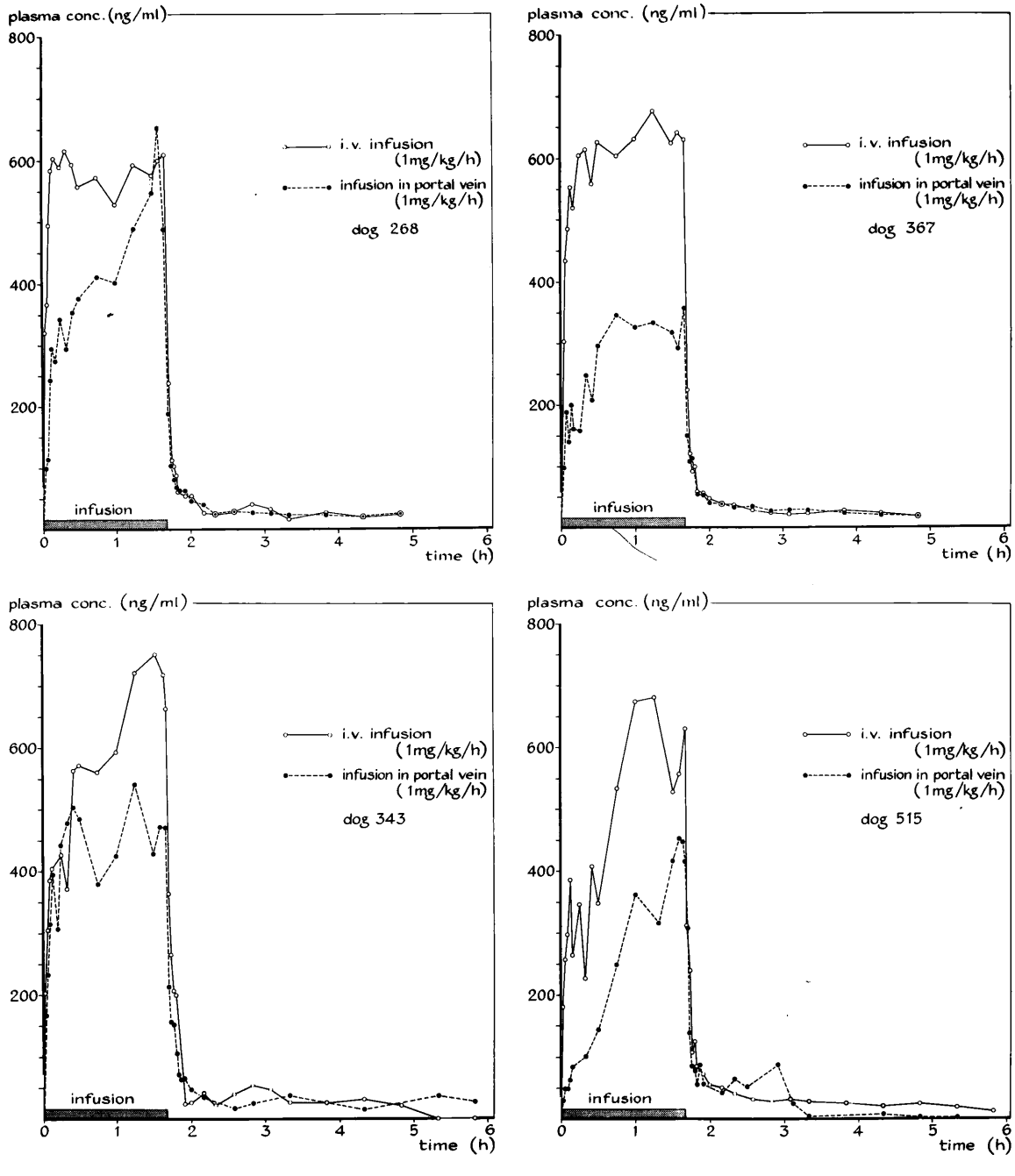


FIG. 2: *Plasma concentrations in dogs after linear infusion of an aqueous solution of thiazinamium methylsulphate in a peripheral vein and in the portal vein.*

The calculated absolute bioavailability F_{abs} after infusion in the portal vein varied from 51.0 to 78.0%, with a mean value of 63.2 ± 13.9(S.D.)%, which implies a "first pass effect" removing 22.0 to 49.0% of the dose before entering the general circulation.
Although it is not allowed to translate findings from an animal experiment to the situation in man, we feel that it does support our conclusion that the liver can remove substantial amounts of thiazinamium cations from the blood in the portal vein. This occurs partly by sulphoxidation with the latter accounting for higher amounts of sulphoxide in the urine than after parenteral administration.

Returning to the studies in humans it is interesting to make an estimation of the meaning of this "first pass effect". Using the ratios between unchanged drug and metabolite found in urine after the intramuscular injection (Table 6 and Table 7 in Chapter 8), where no "first pass effect" occurs, and the quantities of these compounds in urine after oral administration in the same individual, it is possible to correct the value for the bioavailability in the hypothetical case that no sulphoxidation would have occurred during the first liver passage. In this way a quantity of drug is found, which at least must have been absorbed (*N.B.* No correction can be made for the amount of thiazinamium which is possibly removed by the liver and excreted unchanged in the bile, because no information exists about this process after oral administration). To this aim the following equation was used:

$$A = \frac{(Q_{ThSO} - R \cdot Q_{Th}) + Q_{Th}}{Q_{Th}} \cdot F_{rel} \qquad \text{(XVIII)}$$

where:

- A = the amount of thiazinamium cations which must at least have been absorbed from the gastrointestinal tract. Also referred to as "absorption" (% of the dose)
- Q_{Th} = the amount of thiazinamium cations found in urine after oral administration (mol)
- Q_{ThSO} = the amount of thiazinamium sulphoxide cations found in urine after oral administration (mol)
- R = ratio between the amount of thiazinamium cations and thiazinamium sulphoxide cations in urine after intramuscular injection
- F_{rel} = relative bioavailability (% of the dose)

The estimated values for the amount of the drug absorbed (= "absorption") are given in Table 3. The amount extracted during the first liver passage (= "first pass effect") was also estimated and expressed as a percentage of the

amount of the drug absorbed. In six of the ten patients the "first pass effect" accounted for more than 50%, which implies that bioavailability is only half the amount of drug absorbed. In the other patients the "first pass effect" was a little lower. The mean value for all patients was 47.3 ± 25.6% of the absorbed amount.
The impression exists that the variation in absorption is somewhat lower than that in bioavailability.
It is obvious that the variation in the bioavailability is primarily determined by variations in the amount of drug absorbed and in the second instance by variations in the amount of drug eliminated during the first liver passage, as was suggested by WILSON and others (see Chapter 4).
Finally, attention should be paid to the separate group of four patients: D.V., W.B., J.S. and R.W. After intramuscular administration, the amount of sulphoxide found in the urine of these patients was very low: about 2% of the amount that had been systemically available, whereas in the other ten this value was about 32%. Due to a low degree of biotransformation, and therefore a low extent of "first pass effect", high quantities of unchanged drug are found in urine, resulting in a ratio between thiazinamium cations and thiazinamium sulphoxide cations which is almost identical to that found after intramuscular administration. It also results in rather high

TABLE 5: *Patient characteristics and biochemical data (including normal laboratory values; Central Laboratory, University Hospital, Groningen; director Dr. A. GROEN).*

Patient		D.V.	W.B.	J.S.	R.W.	normal laboratory values
Age	(year)	60	24	23	22	
Body weight	(kg)	75	80	86	83	
Height	(m)	1.73	1.93	1.79	1.75	
urea	(mg/100 ml)	31	23	27	27	20 – 40
creatinine	(mg/100 ml)	1.3	0.7	1.0	1.7	0.7 – 1.2
LDH	(U/l)	291	169	233	195	114 – 235
alkaline phosphatase	(m mol U., Bessey)	2.3	1.8	2.6	1.8	0.8 – 3.0
SGOT	(U, Wroblew.)	16	28	33	45	< 35
SGPT	(U, Wroblew.)	17	16	44	67	< 30
bilirubin	(mg %)	–	0.6	–	0.7	< 1.5

bioavailability values, namely of 4.6, 5.0, 12.2 and 5.9% of the dose, with a mean value of 6.9 ± 3.6(S.D.)% of the dose. These values are considerably higher than those found in the other ten patients: 3.6 ± 1.7(S.D.)% of the dose. Because almost no "first pass effect" occurs, the bioavailability value reflects the percentage of real absorption. It is interesting to note that these absorption values are practically identical to those calculated in the other ten patients: 7.1 ± 2.6(S.D.)% of the dose.
It is difficult to explain this low degree of biotransformation. Although it may be due to liver malfunction, no correlation could be found with the outcome of a few conventional laboratory tests on liver function (see Table 5 for biochemical data). In one patient (D.V.) an elevated LDH value was found, in two other patients (J.S. and R.W.) a rather high value for the transaminases SGOT and SGPT was seen, but it is more likely that a lack of oxidating enzymes is responsable for the limited sulphoxidation.

10.3.2. Investigations with a dose of 900 mg

Fig. 3 shows the renal excretion rate and the cumulative renal excretion of thiazinamium cations and thiazinamium sulphoxide cations in patient A.V. Table 2 presents the quantities of these compounds during the time of observation in all fourteen patients.
Generally speaking, the conclusions drawn in the previous section are also valid here. When compared to the study with the lower dose, excretion of both the unchanged drug and the sulphoxide is now slightly lower when expressed as a percentage of the amount which had been available in the systemic circulation. This can possibly be explained by the fact that absorption after a 900 mg dose starts slower and the plasma concentration curve still goes up at the end of our experiment. So, the absorption process is far from complete, and the observation can only be used to get an impression of what is happening at that moment. It is interesting to compare the ratios between unchanged drug and metabolite. In general, there is relatively less sulphoxide when compared to the 300 mg dose, probably due to a more or less retarded absorption. Whereas thiazinamium sulphoxide cations appear later in urine than the unchanged drug, this may be an explanation.
Also a correlation was found between this ratio and the relative bioavailability when compared to the 300 mg dose. In patients F.K., J.A.M., D.M., D.H., A.O. and O.V. after the 900 mg dose the ratio was lower and the relative bioavailability was higher. On the other hand, in patients S.W., K.W., A.V. and E.D. the ratio was higher and bioavailability was lower. This confirms the conclusion that a substantial "first pass effect" occurs. The estimated mean value for the "first pass effect" was not significantly different (Student's t-test; $p > 0.05$) from that found after a dose of 300 mg.

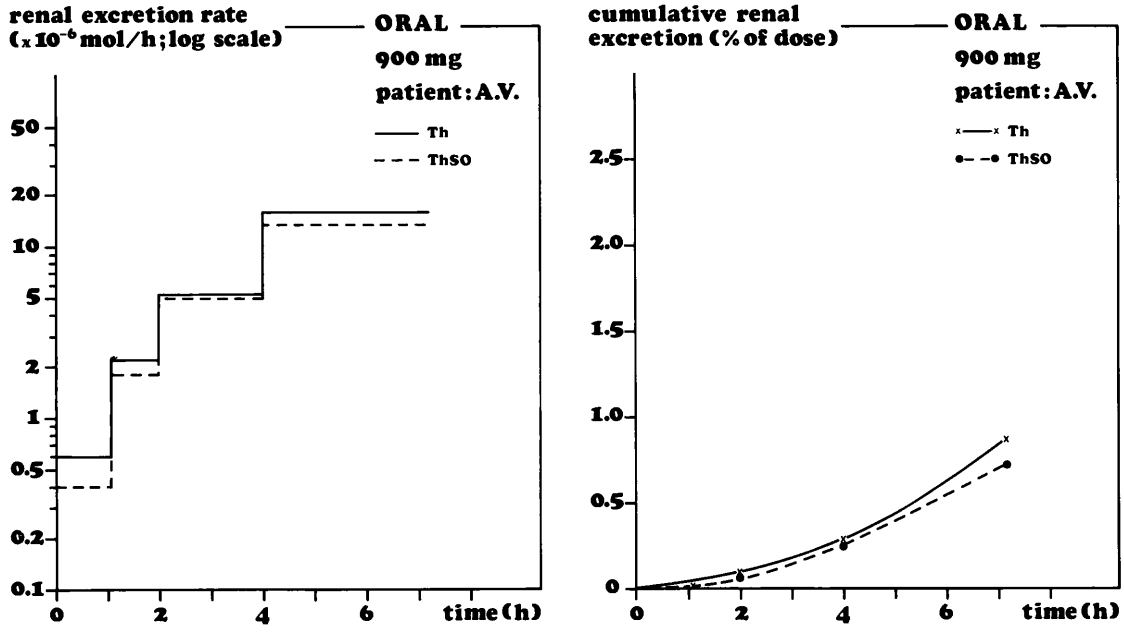


FIG. 3: *Typical examples of renal excretion rate curves (left, on semi-logarithmic scale) and cumulative renal excretion curves (right) of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after oral administration of a dose of 900 mg. Patient: A.V.*

Salivary excretion

Finally, it is of interest to mention the results of a separate study on *salivary excretion* of thiazinamium cations. To this aim the method for the determination of thiazinamium cations in saliva as described in Part II, Section 2.6 was used. In the introduction of that section the motives to undertake this study were mentioned.

Twenty five patients participated in this investigation. They were all under medical supervision with regard to obtaining the optimum thiazinamium dosage regimen as established by determining of the plasma concentrations and measuring the lung function. From these patients blood samples were taken at 90, 150 and 210 min. after oral ingestion of a dose of 300 and (some days later) 900 mg. At the same intervals saliva samples were obtained, if possible. All other conditions were identical to those described in Section 4.2.

The results of this study clearly indicate that the drug diminishes the salivation, in particular in cases of rather good absorption and relatively good bioavailability (*i.e.* rather high plasma concentrations), little or no

saliva was produced. In most of the saliva samples thiazinamium cations could hardly be detected. Only in two patients with very high plasma concentrations (appr. 1000 ng/ml) sufficiently high amounts of the drug could be found in the saliva (appr. 250 ng/ml). However, the number of data was insufficient to see if any correlation between plasma concentration and saliva concentration existed. Moreover, the difficulties in producing sufficient saliva flow led us to the conclusion that it would serve no useful purpose to measure concentrations of thiazinamium in saliva in order to carry out pharmacokinetic studies or in establishing optimum dosage regimens.

Comparison of the results of our study on the biotransformation and excretion of thiazinamium cations in humans after oral administration with data in literature is difficult because the latter is scarce.
HUANG and others (1970) in their study on the fate of promethazine methyliodide (= thiazinamium iodide) only used intraperitoneal injections. These authors suggested that comparison of their results with those obtained by HANSSON and SCHMITTERLÖW (1961) after intramuscular injection of Aprobit® was allowed (see Chapter 7). It is interesting to pay attention again to these two papers in the light of the above mentioned outcome of our investigations. HUANG found that faecal (biliary) excretion was the major route of elimination, whereas HANSSON and SCHMITTERLÖW found that urinary excretion was predominant. However, we suppose that the different routes of administering the drug may be an explanation. HUANG injected intraperitoneally. Drugs which are injected intraperitoneally gain access to the peripheral venous circulation virtually exclusively by way of the hepatic portal system (GIBALDI and PERRIER, 1974). If biotransformation in the rat is not qualitatively different from that in man, in this way the drug is subjected to "first pass effect" and a large proportion of the drug may be eliminated by hepatic clearance. This may result in high biliary excretion of the drug. On the other hand, when giving the drug intramuscularly, as HANSSON and SCHMITTERLÖW did, no "first pass effect" occurs and urinary excretion will predominate.
This influence of the route of drug administration was also clearly seen when comparing the results of our study on intramuscular and oral administration.
Concerning other quaternary ammonium compounds, MCMARTIN and others (1970) report that relatively larger proportions of metabolites were found in the urine after oral rather than after intramuscular administration of guanethidine. This suggests that a significant amount of drug is metabolized while passing through the liver after oral administration. OGUMA and others (1973) found that portal vein infusion of scopolamine-N-butylbromide in the rat results in a substantial increase in the biliary excretion rate and the total amount excreted in the bile as compared to the

values observed when these drugs were administered as an infusion into the femoral vein.

For chlorpromazine, another phenothiazine derivative but not a quaternary ammonium compound, plasma concentrations in rats and dogs obtained after oral and intravenous administration were very different (CURRY, 1971). HOLLISTER and CURRY (1971) found that following the administration of chlorpromazine to human volunteers, the ratio of conjugated to unconjugated metabolites in 24-hours urine was substantially higher following intramuscular injection than after oral doses. Both findings were taken as evidence that chlorpromazine is partially decomposed or metabolized before reaching the general circulation.

"First pass effect" has been reported for several other drugs, and their number is still increasing: *e.g.* acetylsalicylic acid (GIBALDI and FELDMAN, 1969; HARRIS and RIEGELMAN, 1969; ROWLAND and others, 1972), alprenolol (PERRIER and others, 1973), chlorpheniramine (PEATS and others, 1972), imipramine (BICKEL and WEDER, 1968; GRAM and CHRISTIANSEN, 1975; DENCKER and others, 1976; NIAZA, 1976), levodopa (COTLER and others, 1976), lidocaine (BOYES and others, 1971; GUGLER and others, 1975; SHAND and others, 1975), nortriptyline (ALEXANDERSON and others, 1973), paracetamol (COHEN and others, 1974), pentazocine (BURT and BECKETT, 1971), propoxyphene (PERRIER and GIBALDI, 1972; WILSON and others, 1976), propranolol (GIBALDI and others, 1971; SHAND and others, 1975) and terbutaline (CONWAY and others, 1973).

Several general aspects of the "first pass effect" including a pharmacokinetic approach to the phenomenon have been given by GIBALDI and FELDMAN (1972), GIBALDI and PERRIER (1974), ROWLAND (1972) and ROWLAND and RIEGELMAN (1973).

Conclusion

Although after oral administration of thiazinamium methylsulphate the renal clearance was also found to be very quick, the excretion process was still happening at the end of the observation (t = 480 min.). Individual renal clearance values were calculated and the mean value was found to be 256 ± 136 (S.D.) ml/min., which confirmed the expected occurrence of an active excretion process. Hepatic clearance (biotransformation and biliary excretion of unchanged drug) was calculated and accounted for 537 ± 495(S.D.) ml/min. A correlation was found between the value of hepatic clearance and the amount of sulphoxide in urine, whereas hepatic clearance and bioavailability were negatively correlated. The ratio between unchanged drug and metabolite in urine after oral administration was substantially different (about 1 : 0.94) from that found after intramuscular injection (about 1 : 0.20) in the same patients. The findings can be explained by

assuming that a substantial "first pass effect" occurs. More evidence for such a phenomenon was found in the dog by comparison of plasma concentrations obtained after intravenous infusion and infusion in the portal vein.

Bioavailability values were corrected for "first pass effect" resulting in figures for the amount of drug which must at least have been absorbed from the gastrointestinal tract. Absorption was found to be about 7% of the dose during the first seven hours of the study, which implies that about half of the absorbed amount of thiazinamium cations was eliminated from the blood during the first liver passage. The values for absorption were confirmed by the results obtained in patients with a negligible amount of sulphoxide in the urine, the latter being considered as an indication of little or no "first pass effect".

References

ALEXANDERSON, B., O. BORGÅ and G. ALVÁN (1973), *Europ. J. Clin. Pharmacol. 5,* 181

BICKEL, M. H. and H. J. WEDER (1968), *Life Sci. 7,* 1223

BOYES, R. N., D. B. SCOTT, P. J. JEBSON, M. J. GODMAN and D. G. JULIAN (1971), *Clin. Pharmacol. Ther. 12,* 105

BURT, R. A. P. and A. H. BECKETT (1971), *Br. J. Anaest. 43,* 427

COHEN, G. M., O. M. BAKKE and D. S. DAVIES (1974), *J. Pharm. Pharmacol. 26,* 348

CONWAY, W. D., S. M. SINGHVI, M. GIBALDI and R. N. BOYES (1973), *Xenobiotica 3,* 813

COTLER, S., A. HOLAZO, H. G. BOXENBAUM and S. A. KAPLAN (1976), *J. Pharm. Sci. 65,* 822

CURRY, S. H. (1971), *Proc. Roy. Soc. Med. 64,* 285

DENCKER, H., S. J. DENCKER, A. GREEN and A. NAGY (1976), *Clin. Pharmacol. Ther. 19,* 584

ETRILLARD, E. (1976), *Personal Communication,* from the Laboratories of SPECIA, Rhône Poulenc, Paris, France

GIBALDI, M., R. N. BOYES and S. FELDMAN (1971), *J. Pharm. Sci. 60,* 1338

GIBALDI, M. and S. FELDMAN (1969), *J. Pharm. Sci. 58,* 1477

GIBALDI, M. and S. FELDMAN (1972), *Europ. J. Pharmacol. 19,* 324

GIBALDI, M. and D. PERRIER (1974), in: *"Drug Metabolism Reviews"* Vol. 3, p. 185 (F. J. di Carlo, Ed.), Marcel Dekker, Inc., New York

GRAM, L. F. and J. CHRISTIANSEN (1975), *Clin. Pharmacol. Ther. 17,* 555

GUGLER, R., P. LAIN and D. L. AZARNOFF (1975), *J. Pharmacol. Exp. Ther. 195,* 416

HANSSON, E., and C. G. SCHMITTERLÖW (1961), *Arch. Int. Pharmacodyn. 131,* 309

HARRIS, P. A. and S. RIEGELMAN (1969), *J. Pharm. Sci. 58,* 71

HOLLISTER, L. E. and S. H. CURRY (1971), *Res. Commun. Chem. Pathol. Pharmacol. 2,* 330

HUANG, C. L., J. A. YEH and S. Y. HSU (1970), *J. Pharm. Sci. 59,* 772

MCMARTIN, C., R. K. RONDEL, J. VINTER, B. R. ALLAN, P. M. HUMBERSTONE, A. W. D. LEISHMAN, G. SANDLER and J. L. THIRKETTLE (1970), *Clin. Pharmacol. Ther. 11,* 423

NIAZA, S. (1976), *J. Pharm. Sci. 65,* 1063

OGUMA, T., T. MARAMATSU, T. IGA, T. FUWA, S. AWAZU and M. HANANO (1973), *Chem. Pharm. Bull. 21,* 1554

PEATS, E. A., M. JACKSON and S. SYMCHOWICZ (1972), *J. Pharmacol. Exp. Ther. 180,* 464

PERRIER, D. and M. GIBALDI (1972), *J. Clin. Pharmacol. 12,* 449

PERRIER, D., M. GIBALDI and R. N. BOYES (1973), *J. Pharm. Pharmacol. 25,* 256

RITSCHEL, W. A. (1976), in: *"Handbook of Basic Pharmacokinetics"*, Drug Intelligence Publication, Hamilton, Illinois 62341, U.S.A.

ROWLAND, M. (1972), *J. Pharm. Sci. 61,* 70

ROWLAND, M. and S. RIEGELMAN (1973), *J. Pharmacokin. Biopharm. 1,* 419

ROWLAND, M., S. RIEGELMAN, P. A. HARRIS and S. D. SHOLKOFF (1972), *J. Pharm. Sci. 61,* 379

SHAND, D. G., D. M. KORNHAUSER and G. R. WILKINSON (1975), *J. Pharmacol. Exp. Ther. 195,* 424

WILSON, J. T., G. F. ATWOOD and D. G. SHAND (1976), *Clin. Pharmacol. Ther. 19,* 264

11

Biotransformation and excretion after rectal administration in man

Contents

11.1. Introduction

The fate of thiazinamium cations after rectal administration of the drug contained in a lipophilic and in a hydrophilic base was investigated in man. The amount of the unchanged drug and metabolite excreted in the urine was compared to the values found after intramuscular injection of the drug.

11.2. Materials and methods

11.2.1. Investigations with Witepsol H-15 suppositories

Subjects and conditions

The seven patients already described in Section 3.2.2.2 participated in this study. The results of one patient (H.H.) were omitted because of insufficient data.

Urine was voided every hour if possible, up to seven hours following the administration of the drug. Five of the patients (see Table 1) collected additional fractions of urine up to 24 hours after giving the dose.
All other conditions, materials and methods were identical to those described in Section 5.2.1.

11.2.2. Investigations with polyethylene glycol suppositories

Patients, conditions, materials and methods were the same as described in Sections 3.2.2.2 and 5.2.2.

11.3. Results and discussion

11.3.1. Investigations with Witepsol H-15 suppositories

Fig. 1 presents the renal excretion rate and the cumulative renal excretion of thiazinamium cations and thiazinamium sulphoxide cations in patient G.L. In Table 1 the quantities of these compounds excreted during the time of the experiment are given.
As after all previously described routes of administration, the excretion of thiazinamium cations appeared to be very fast. The total amount of the unchanged drug excreted during the first seven hours of the study depended, among other things, on the absorption rate and the period within which the absorption process was finished (see plasma concentration-time curves in Chapter 5, Fig. 3a). In one patient (W.B.) with a very low absorption, excretion was completed within seven hours. The other patients had already excreted in seven hours 80 to 94% of the total amount excreted in 24 hours. The amount of the unchanged drug excreted over a period of 24 hours varied from 28.7 to 96.9% of the calculated value of bioavailability. It should be noted, however, that the value of 96.9% (patient W.B.) is much higher than those obtained in the other patients and that W.B. may represent a special case. Omitting this value the excretion varied between 28.7 and 47.9% of the calculated bioavailability, with a mean value of 38.8 $\pm$ 8.6(S.D.)%. It is interesting to compare the individual values for the excreted amount of the unchanged drug expressed as percentage of the bioavailability with those obtained after intramuscular injection, where

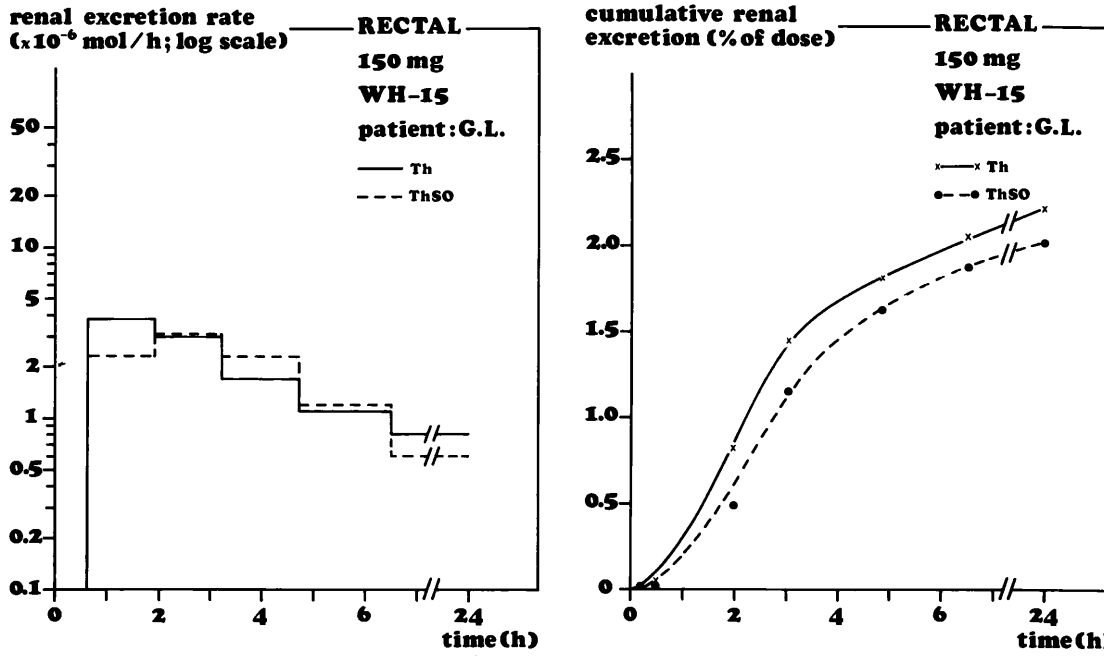


FIG. 1: *Typical examples of renal excretion rate curves (left, on semi-logarithmic scale) and cumulative renal excretion curves (right) of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after rectal administration of a dose of 150 mg. Suppository base: Witepsol H-15. Patient: G.L.*

bioavailability was found to be 100% (see Chapter 3). Such a comparison was possible in four patients, yielding the following values for rectal and intramuscular administration respectively: G.L. 35.1 and 44.4%; P.H. 28.7 and 30.3%; W.M. 47.9 and 35.1% and M.F. 43.4 and 29.6%. These values show that there exists in general an acceptable correlation between the excretion after administering the drug either rectally or intramuscularly. With regard to the renal excretion of thiazinamium sulphoxide cations Fig. 1 shows that the excretion rate is almost identical to that of the unchanged drug. The amount of thiazinamium sulphoxide cations found in the urine after rectal administration expressed as percentage of the bioavailability was substantially higher than after intramuscular injection. For patients G.L., P.H., W.M. and M.F. a mean value of 30.4 ± 9.2 (S.D.)% was found. The ratio between the unchanged drug and metabolite varied from 1 : 0.47 to 1 : 1.67 (W.B.) resulting in a mean value of 1 : 1.01. Omitting the value for W.B. a mean ratio of 1 : 0.78 was found, which is comparable to the ratio found after oral administration of the drug. These findings strongly suggest that after rectal administration a substantial "first pass effect" occurs, being of

TABLE I: *Cumulative urinary excretion of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after rectal administration of a dose of 150 mg (= 47.5 × 10^{-6} mol) in a Witepsol H-15 suppository. For estimation of "absorption" see text.*

Patient		G.L.	P.H.	W.M.	W.B.	M.F.	T.J.[1]	mean ± S.D.[2]
Th	(× 10^{-6} mol)	10.5	9.0	24.9	3.0	11.9	6.5	11.9 ± 8.0
Th	(% of dose)	2.2	1.9	5.3	0.6	2.5	1.4	2.5 ± 1.7
Th	(% of F_{rel})	35.1	28.7	47.9	96.9	43.4	33.3	55.9 ± 16.5
ThSO	(× 10^{-6} mol)	9.6	8.4	22.0	5.0	5.6	7.4	10.4 ± 6.7
ThSO	(% of dose)	2.0	1.8	4.6	1.0	1.2	1.6	2.1 ± 1.5
ThSO	(% of F_{rel})	32.1	26.7	42.2	161.5	20.4	38.0	56.6 ± 59.2
Th + ThSO	(× 10^{-6} mol)	20.1	17.4	46.9	8.0	17.5	13.9	22.0 ± 14.7
Th + ThSO	(% of dose)	4.2	3.7	9.9	1.6	3.7	3.0	4.0 ± 3.1
Th + ThSO	(% of F_{rel})	67.2	55.4	90.1	258.4	63.8	71.3	106.9 ± 85.6
Th : ThSO		1 : 0.92	1 : 0.93	1 : 0.88	1 : 1.67	1 : 0.47	1 : 1.14	1 : 1.01
$(F_{rel})_{tot}$	(% of dose)	6.6	6.8	11.6		5.8		7.7 ± 2.6
"Absorption"	(% of dose)	11.6	11.4	18.5		7.0		12.1 ± 4.8
"First pass effect" (% of "absorption")		43.1	40.4	37.3		17.1		34.5 ± 11.8

[1] = until 390 min. after administration

[2] = mean of data of five patients (T.J. omitted).

the same degree as that following oral administration (JONKMAN and others, 1976). This would imply that thiazinamium cations do not bypass the first liver passage when administered rectally. This conclusion is rather surprising because - although there is no concrete evidence - it is generally believed that after rectal administration drugs will enter the systemic circulation for the greater part without initial passage through the liver, provided that the suppository does not reach the higher parts of the rectum (see Section 5.1). Not withstanding the fact that this hypothesis has been widely accepted, some authors have questioned it. For example GIBALDI and PERRIER (1974) discussed the two possibilities of entering the venous circulation after rectal application, namely directly or by way of the hepatic portal system. The authors came to the following conclusion: "While there is some debate as to which category rectal administration falls into, it is the authors' view that this is essentially a hepatic route". However no exact evidence for this opinion was given.

In order to explain the occurrence of a "first pass effect" after rectal administration of thiazinamium cations, it must be assumed that this drug enters the systemic circulation almost exclusively by way of the smaller

rectal vessels, draining in the portal vein, just as in the case of oral administration. It was pointed out in Section 5.1 that only the veins of the higher part of the rectum, the superior rectal veins (*vena rectalis cranialis*), drain directly in the portal vein (*vena porta*). So, the drug must have been transported to a large extent by way of the superior rectal veins. If part of the drug is absorbed in the inferior rectal veins (*vena rectalis caudalis*) and/or the middle rectal veins (*vena rectalis medialis*) it seems likely that it may afterwards enter the superior rectal veins by way of the numerous connections (anastomoses) between the latter and the previously mentioned veins (see also Fig. 1 in Section 5.1).

Another possibility is that the (molten) suppository rapidly reaches the higher parts of the rectum, resulting in direct absorption into the superior rectal veins.

In order to get a quantitative impression of the meaning of the "first pass effect" after rectal administration, we have estimated the value for the amount of drug which must at least have been absorbed ("absorption") in four of the patients by means of equation XVIII (Section 10.3.1.). The obtained values are given in Table 1. A mean value of 12.1 ± 4.8(S.D.)% of the dose was found. The sulphoxidation during the first passage through the liver - expressed as percentage of the amount of drug absorbed - appeared to vary from 17.1 to 43.1% (mean 34.5 ± 11.8(S.D.)%). It is interesting to compare these values with those obtained after oral administration *e.g.* the 300 mg dose. Although the mean value is lower when compared to oral administration, no significant difference could be found in the degree of "first pass effect" occurring after oral or rectal administration of thiazinamium cations (Student's t-test, $p > 0.05$). It should be realized however that we are dealing here with two different groups of patients and that the number of patients is limited.

11.3.2. Investigations with PEG suppositories

The urinary excretion data for both thiazinamium cations and thiazinamium sulphoxide cations obtained after administration of the drug in PEG suppositories are given in Table 2. In almost all cases the quantities excreted are very low and a reliable calculation of the amount of drug that at least must have been absorbed and of the degree of "first pass effect" is hardly possible. However, the observations suggest that our conclusion that thiazinamium cations are subjected to "first pass effect" after rectal administration can also be applied here.

TABLE 2: *Cumulative urinary excretion of thiazinamium cations (= Th) and thiazinamium sulphoxide cations (= ThSO) after rectal administration of a dose of 150 mg (= 47.5 × 10^{-6} mol) in a PEG suppository.*

Patient		G.L.	P.H.	W.M.	W.B.	M.F.	T.J.	mean ± S.D.
Th	(× 10^{-6} mol)	0.8	0.5	0.5	0.4	2.0	0.7	0.8 ± 0.6
Th	(% of dose)	0.1	0.1	0.1	0.1	0.4	0.1	0.1 ± 0.1
Th	(% of F_{rel})	–	3.4	25.0	–	–	14.3	–
ThSO	(× 10^{-6} mol)	0.5	0.5	0.8	0.2	1.1	0.7	0.6 ± 0.3
ThSO	(% of dose)	0.1	0.1	0.2	0.1	0.2	0.2	0.1 ± 0.1
ThSO	(% of F_{rel})	–	3.4	40.0	–	–	14.3	–
Th + ThSO	(× 10^{-6} mol)	1.3	1.0	1.3	0.6	3.1	1.4	1.5 ± 0.9
Th + ThSO	(% of dose)	0.2	0.2	0.3	0.1	0.6	0.3	0.3 ± 0.2
Th + ThSO	(% of F_{rel})	–	6.8	65.0	–	–	28.3	–
Th : ThSO		1 : 0.6	1 : 1	1 : 1.6	1 : 0.5	1 : 0.5	1 : 1	1 : 1

– = reliable estimation impossible

Conclusion

After rectal administration of thiazinamium methylsulphate contained in a Witepsol H-15 suppository, the renal excretion appeared to be very quick. Within the first seven hours of the study about 85% of the totally excreted amount of unchanged drug was found in the urine. In 24 hours about 38.8% of the dose was excreted in the unchanged form, whereas about 30.4% was excreted as sulphoxide. So, the ratio between unchanged drug and sulphoxide in urine was considerably different (about 1 : 0.78) from that found after intramuscular injection in the same individuals (about 1 : 0.24). This observation can be explained by assuming that after absorption a substantial amount of the drug is subjected to biotransformation and elimination by the liver during the first passage ("first pass effect"). The estimated value for the amount of drug that must at least have been absorbed was about 12.1% of the dose (*N.B.* The absorption process was finished during the time of the experiment). On the other hand the value for the bioavailability was about 7.7%. These calculations imply that about 34.5% of the absorbed amount of drug is subjected to biotransformation during the first liver passage and will enter the peripheral venous circulation as the inactive sulphoxide. The values are of the same order of magnitude as those found after oral administration.

Our findings point out that rectal administration of thiazinamium methylsulphate contained in a Witepsol H-15 suppository has no major advantages over oral administration from a biopharmaceutical point of view. After rectal administration the peak plasma concentration does appear earlier, which is the only clinical advantage. But the total absorption and the extent of the "first pass effect" are of the same order of magnitude, resulting in an almost equal bioavailability.

References

GIBALDI, M. and D. PERRIER (1974), in: *"Drug Metabolism Reviews"*, Vol. 3, p. 185 (F. J. di Carlo, Ed.), Marcel Dekker, Inc., New York

JONKMAN, J. H. G., L. E. VAN BORK, J. WIJSBEEK, R. A. DE ZEEUW, N. G. M. ORIE and H. L. M. COX (1976), *J. Pharm. Pharmacol. 28*, Suppl. 56P

12

Correlation between plasma concentration and heart rate

Contents

12.1. Introduction

Although the correlation between plasma concentrations of thiazinamium cations and clinical effect is primarily the subject of the thesis of L. E. VAN BORK, we want to pay some attention here to the question of how far monitoring pharmacological effects can be of help in determining of the bioavailability.

In the last decade, determination of plasma concentrations of drugs has assumed wide proportions. Not only for toxicological aims, but especially for the determination of pharmacokinetic parameters and also as a resource in therapeutics.

An example has been given in the previous chapters.

On the other hand, in some cases where it was hard to measure plasma concentrations of the drug, it has been suggested that it may be possible to determine the bioavailability of a drug by quantitatively monitoring an easily measurable pharmacological (side-)effect. An example was given by SMOLEN and others (1975) for chlorpromazine, using pupilometry.

Pharmacological data sampling has the advantage over direct assay data in that it can be recorded more frequently and continuously by noninvasive methods. In addition the information is immediately available and does not require expensive apparatus. Another advantage can be that the measurements take place in the biophase (tissue or peripheral compartment).

A prerequisite for measuring pharmacological effects is that there is a good

correlation between plasma concentration and effect, as, for example, has been demonstrated for some anticonvulsive drugs, anti-arithmic drugs, theophylline, amitriptyline, nortriptyline and with some antibiotics. Lower accuracy, however, may be a disadvantage of the method of monitoring pharmacological effects.

We will discuss the usefulness of monitoring the changes in the heart rate after the administration of thiazinamium methylsulphate, in relation to the calculation of the relative bioavailability of the drug. With this aim in mind, we will deal with the results of the study involving fourteen patients, in which two oral doses were compared with intramuscular administration in the same patients.

12.2. Materials and methods

Estimation of the relative bioavailability by monitoring the heart rate

The heart rate was measured using the methods described in Section 2.2. The value obtained before the drug administration was considered as the "zero" value of the heart rate-time curve. During the investigations the heart rate was always measured two minutes before a blood sample was taken. The curves of the heart rate-time after the oral dose and the intramuscular administration were drawn on the same scale on a standard type of paper. The areas under the heart rate-time curves were determined by cutting and weighing. The relative bioavailability of the oral dose, when compared to the intramuscular administration was calculated as described in Chapter 1 for the areas under the plasma concentration-time curves.

The subjects and all circumstances, materials and methods were identical to those described in Sections 3.3.3.2, 4.2.1, 4.2.2, 9.2.3.2, 10.2.1 and 10.2.2.

12.3. Results and discussion

In Fig. 1 the curves are given for both the plasma concentrations and the changes in the heart rate *versus* time as they were observed after an intramuscular injection of a dose of 25.0 mg in patient F.K. In Fig. 2 such curves are given for oral administration of 300 mg and 900 mg in the same patient.
These curves are typical examples of the curves found for other patients. They show that a correlation exists between the plasma concentration and the heart rate especially in cases of higher plasma concentrations. The increase of the heart rate with increasing plasma concentration of thiazinamium cations is due to the anticholinergic activity of the latter and can be seen as a side-effect (see Part I, Section 6.2.2.).
As Fig. 1 shows, the peak in the heart rate-time curve appeared a little later than the peak in the plasma concentration-time curve, but it is only a matter of some minutes. This time-lag may indicate the time necessary for penetration to and occupation of the acetylcholine receptors.

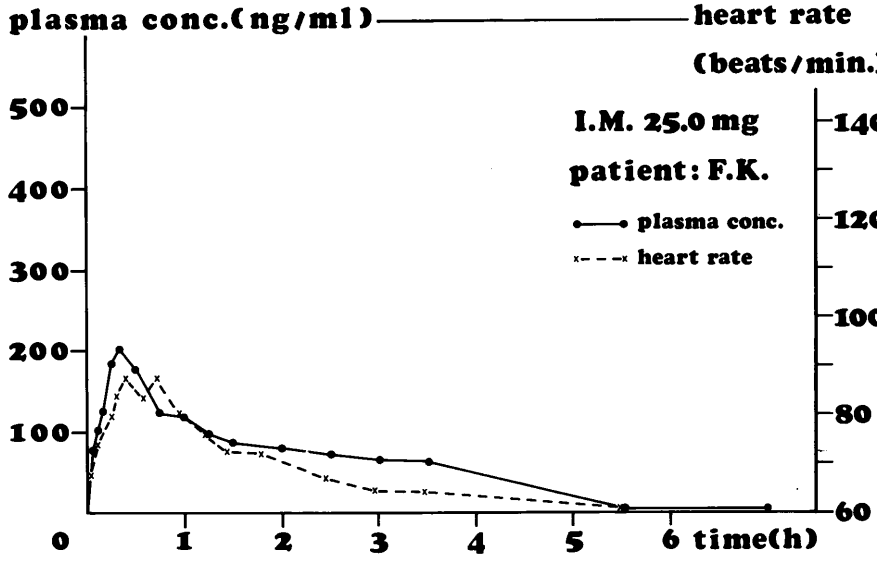
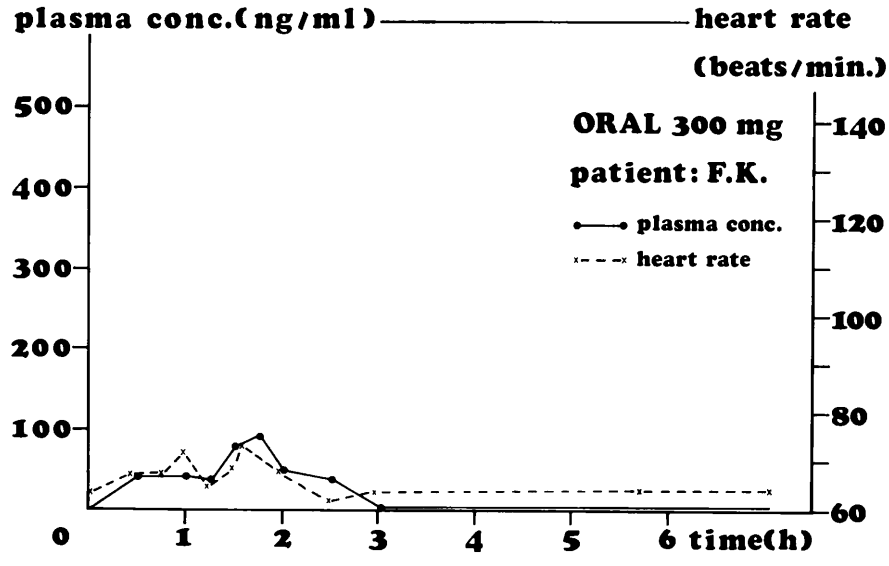


FIG. 1: *Typical examples of the plasma concentration-time curves and the heart rate-time curves obtained after intramuscular administration of a dose of 25.0 mg. Patient: F.K.*



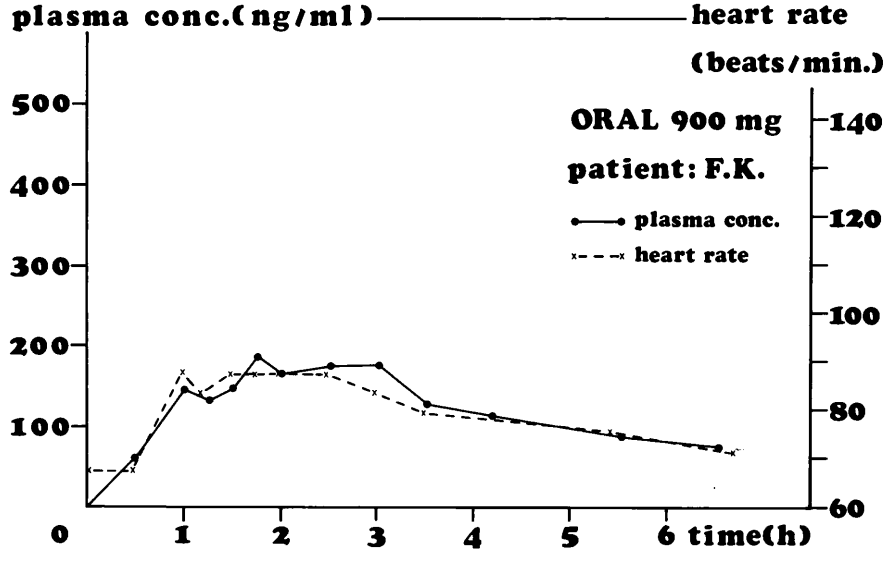


FIG. 2: *Typical examples of the plasma concentration-time curves and the heart rate-time curves obtained after oral administration of a dose of 300 mg (above) and 900 mg (below) in the same patient (F.K.).*

Rather substantial interindividual differences were found between the increase of the heart rate compared to the plasma concentration at a certain point of time. These differences in effect were possibly due to differences in anatomy and physiology of the cardiovascular system of the patients. Assuming that we are dealing with the linearly increasing part of the plasma concentration-effect curve (see thesis L. E. VAN BORK), we decided to investigate how far the heart rate-time curves could be used in order to calculate the relative bioavailability of an orally administered dose compared to an intramuscular injection in the same patient. The results of these calculations are given in Fig. 3.

After giving a dose of 300 mg the mean value for the relative bioavailability was 5.2 ± 4.2(S.D.)% of the dose when calculated by monitoring the heart rate and 5.5 ± 2.7(S.D.)% of the dose when calculated by measuring plasma concentrations of the drug. By comparing the individual values by means of the paired t-test, no significant difference could be found between these values ($p > 0.05$). However, the diagram in Fig. 3 shows that there are certain differences. But, it should be realized, that the method of monitoring the heart rate has a limited accuracy, which can easily lead to a deviation of plus or minus 4 beats/min. This value can be of considerable importance in the cases of low plasma concentrations (as often found after a 300 mg dose) where in general, only small increases of the heart rate are observed. Moreover, wrong registration of the heart rate at the moment just before the drug administration (the "zero" value) can lead to a considerable deviation in the area under the curve, especially after giving a lower dose. This can result in a deviation in the value of the bioavailability obtained in this way.

After administration of a dose of 900 mg the mean value for the relative bioavailability was 5.5 ± 3.5(S.D.)% of the dose when calculated by monitoring the heart rate and 5.4 ± 3.4(S.D.)% of the dose when calculated by measuring the plasma concentrations. Also these values are not significantly different (paired t-test; $p > 0.05$). The diagram in Fig. 3 shows that in 12 of the 14 patients a good correlation was found between the values of the relative bioavailability calculated by the two methods. In the two other patients some differences were found. The fact that better correlations are found after giving the higher dose can probably be explained by the higher accuracy of the methods of determining the bioavailability, which is especially valid for the method which uses the changes in the heart rate. However, we feel that our results indicate that by simply measuring the heart rate it is possible to get an impression of the order of magnitude of the bioavailability of thiazinamium cations after oral administration. Investigations after rectal administration of the drug have confirmed this conclusion.

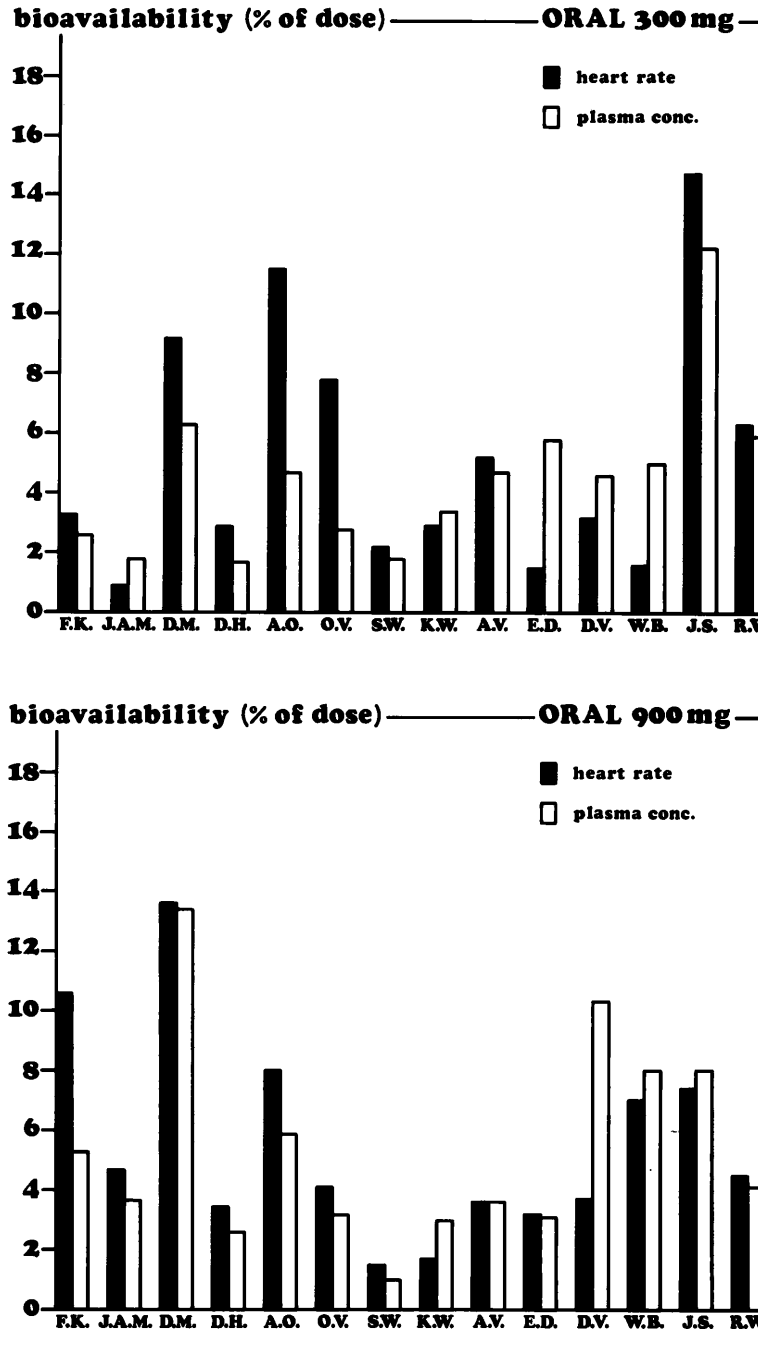


FIG. 3: *Comparison of the relative bioavailability calculated by means of the plasma concentration-time curves (white bars) and by means of the heart rate-time curves (black bars). The figures are obtained after oral administration of a dose of 300 mg (above) and 900 mg (below) in the same patients.*

Conclusion

After the administration of thiazinamium methylsulphate a correlation was found in general between the plasma concentrations of the drug and the heart rate in humans. This correlation was better when dealing with higher plasma concentrations and greater changes in the heart rate.
It appeared to be possible to get an impression of the order of magnitude of the relative bioavailability of the drug after oral administration by calculating it from the areas under the heart rate-time curves obtained after oral and intramuscular administration. The higher dose gave a greater degree of accuracy with this method.

Reference

SMOLEN, V. F., H. R. MURDOCK and E. J. WILLIAMS (1975), *J. Pharmacol. Exptl. Ther. 195*, 404

13

Whole body autoradiography

Contents

13.1. Introduction

The distribution of ^{35}S-ringlabelled thiazinamium methylsulphate has been studied by means of whole body autoradiography in a monkey and in mice. The experiments were performed on regional distribution that could add to the pharmacokinetic data of thiazinamium methylsulphate obtained from plasma concentration data described in the previous chapters.
Whole body distribution was investigated in the monkey, being a primate. Special attention was paid to a possible passage through the "blood-brain barrier" and to accumulation in different organs and regions. The distribution was studied under "steady state" conditions obtained by linear infusion. Plasma samples were analyzed for thiazinamium cations by means of a chemical method and for total radioactivity by means of a radiochemical assay in order to check in how far the radioactivity observed in the autoradiograms was caused by the unchanged drug.
A study has been undertaken in mice to obtain information concerning the rate of release from the injection site and the rate of appearance of the drug in different organs after intramuscular injection in the thigh muscles. The animals were killed at different time periods after the injection varying from two minutes to six hours.

Pregnant mice were studied for the rate and extent of placenta transfer for thiazinamium methylsulphate.
Finally, it was interesting to investigate the specific influence of the quaternary ammonium group on the distribution pattern of the drug. To obtain this information all experiments were repeated in an identical way with ^{35}S-labelled promethazine (as hydrochloride), the tertiary analogue of thiazinamium cations.

13.2. Materials and methods

Generally speaking the distribution of radioactivity was studied following the whole body autoradiography method of ULLBERG (1954, 1958), also described by VAN DER KLEIJN (1969).
All the experiments were performed at the Department of Clinical Pharmacy, St. Radboud Hospital, Catholic University, Nijmegen and were supervized by DR. E. VAN DER KLEIJN.

13.2.1. Investigations in a monkey

Animals and conditions

In the thiazinamium study a female squirrel monkey (Saimiri) weighing 550 g had been starved overnight and was anesthetized by intravenous injection of pentobarbital sodium (30 mg/kg). Anesthesia was maintained by inhalation of oxygen, nitrous oxide and fluothane. The heart rate and body temperature were monitored. Linear infusion was given through a catheter into the femoral vein. Infusion time was 60 min. During the infusion period blood samples of 0.6 ml were taken by means of a second catheter in the opposite femoral artery at 4, 9, 19, 29, 44 and 59 min. after starting the infusion. The blood was collected in heparinized polyvinyl-chloride tubes and it was immediately centrifugated. The plasma was stored at $-20°$ C until it was analyzed.
At the end of the infusion period the animal was killed under a deeper anesthesia by submersion into iso-pentane, cooled with solid carbon dioxide ($-80°$ C).

Dosage form

The ^{35}S-labelled thiazinamium methylsulphate was prepared at the Radiochemical Centre, Amersham, England and kindly supplied by SPECIA, Rhône Poulenc, Paris, France. The substance was found to be at least 99.9% radiochemically pure by thin-layer chromatography. Specific activity at the time of the experiment was 8.1 mCi/mmol (= 19.6 μCi/mg). The drug was dissolved in a sterile physiological salt solution. The total volume of the infusion solution was 4.4 ml containing 21.8 mg thiazinamium methylsulphate which corresponds to 39.7 mg/kg. The dose of radioactivity was 428.5 μCi (= 0.8 μCi/g of body weight).

Autoradiography

The distribution of radioactivity was studied as described by VAN DER KLEIJN (1969). Sagittal sections of 30 μm were made with a P.M.V. "freeze"-microtome with 45 cm sledge (Palmstyrnas Mekaniska Verkstad, Stockholm, Sweden). At a temperature of −15 °C sections were taken across the whole animal untill all organs of interest were collected.
All other conditions were identical to those described in the paper mentioned.

Determination of thiazinamium cations in plasma, urine and bile

The methods described in Part II, Chapter 2 were used.

Determination of thiazinamium sulphoxide cations in urine and bile

These analyses were performed as described in Part II, Chapter 3.

Radiochemical analysis

Plasma samples of 50 μl each were mixed with 10 ml of Instagel® (Packard Instrument Company Inc., Downers Grove, Ill., U.S.A.) and the radioactivity was estimated by means of a Packard Tri Carb 3380 AAA Liquid Scintillation Spectrometer using the method of external standardization. The samples of urine and bile were discoloured by standing overnight with 5 drops of hydrogen peroxide (30%) at 37° C. From each sample a 50 μl fraction was treated as described above. The urine and bile samples were also submitted to thin-layer chromatography as described in Part II,

Chapter 3 and the amount of radioactivity of the thiazinamium and thiazinamium sulphoxide spots was determined with an L.B. 2723 Berthold Dünnschicht Scanner II.
In some of the other organs (see Table 1) radioactivity was determined as follows: the organs were isolated from the remaining part of the monkey. A part of the organ (100 to 400 mg) was rinsed with water and dried and an accurately weighed fraction was transferred to a counting vial. Then 1.0 ml of NCS® solution (NCS Solubilizer for Liquid Scintillation Counting, Amersham/Searle, U.S.A.) was added and the mixture was heated in a water bath at 45° C for 12 hours. After cooling to room temperature, 5 drops of hydrogen peroxide (30%) were added and the heating was continued for another hour. Then 20 ml of an Instagel®/ hydrochloric acid mixture 9 : 1 was added and counting took place as described above.

13.2.2. Investigations in mice

Animals and conditions

Adult male Swiss mice weighing about 30 g and pregnant mice about two days before delivery weighing about 50 g were used. All the animals were starved overnight before the experiment. Injections were made intramuscularly in the right thigh. The mice were killed 2, 5, 10, 15, 30, 60, 120, 240 en 480 min. after injection by submersion into iso-pentane, cooled with solid carbon dioxide (− 80° C).
For the pregnant mice the survival periods were 10, 30, 120 and 240 min.

Autoradiography was performed as described above.

Dosage form

The drug substance was the same as described in Section 13.2.1. The concentration of thiazinamium methylsulphate in the injection solution was 7.5 mg/ml. The injection volume was about 0.05 ml but was adapted so that all animals received a dose of 0.015 mg/g (= 0.3 μCi/g).

13.3. Results and discussion

13.3.1. Investigations in a monkey

Fig. 1 presents the plasma concentration-time curve obtained during the experiment, determined by chemical analysis of the amount of thiazinamium cations and also by counting the total radioactivity (both expressed as thiazinamium methylsulphate). The high degree of correlation between the two curves suggests that apart from the unchanged drug only a very small amount of other ^{35}S containing compounds (*e.g.* metabolites) are present in the plasma.
Generally speaking this implies that almost all the observed radioactivity corresponds to the unchanged thiazinamium cations (an exception should be made for excretion organs and their excretion products, see below).
The amount of radioactivity (calculated as thiazinamium methylsulphate) found by tissue analysis in the different organs is given in Table 1.

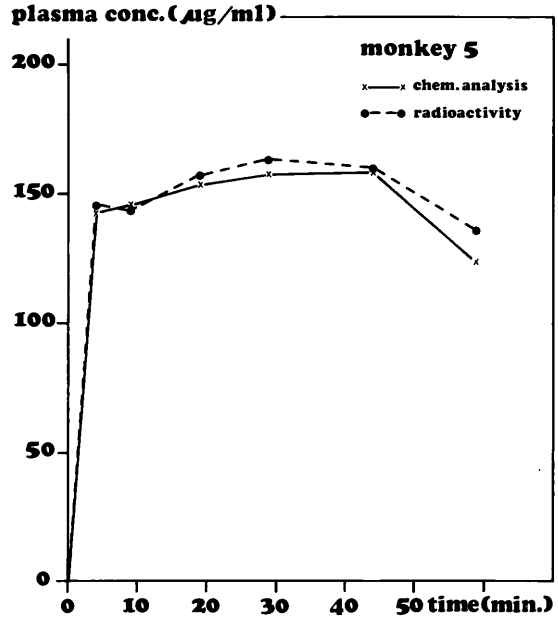


FIG. 1: *Plasma concentrations in a monkey as obtained after linear infusion of ^{35}S-thiazinamium methylsulphate until "steady state". The amounts of drug are determined both by chemical analysis (expressed as µg/ml) and by counting the total radioactivity present in the plasma (via specific activity calculated as thiazinamium methylsulphate and expressed as µg/ml).*

TABLE I: *Tissue analysis in a monkey after a linear infusion of ^{35}S-thiazinamium methylsulphate until "steady state."*

organ	total radioactivity[1] $\times 10^{-9}$ mol	ratio plasma: tissue	chemical analysis Th $\times 10^{-9}$ mol	ThSO $\times 10^{-9}$ mol
cerebrum	appr. 0.1	1 : 0.0003		
cerebellum	appr. 0.1	1 : 0.0003		
medulla oblongata	appr. 0.1	1 : 0.0003		
spinal cord	appr. 0.1	1 : 0.0003		
plasma	302	–		
lung	41	1 : 0.14		
liver	2248	1 : 7.44		
gall bladder	2040	1 : 6.76	987	1083
spleen	56	1 : 0.19		
stomach	70	1 : 0.23		
duodenum	75	1 : 0.25		
ileum	3040	1 : 10.07		
kidney	2801	1 : 9.27		
urine	707	1 : 2.34	542	29

[1] = calculated as thiazinamium methylsulphate per g of tissue or ml of bodyfluid.

Autoradiograms of ^{35}S-thiazinamium methylsulphate in the squirrel monkey are given in Figs. 2, 3, 4 and 5a.
The pictures show that the drug had been distributed unequally through the body (Figs. 2 and 3).
High accumulations of radioactivity were observed in the two organs that are mainly responsable for the elimination of thiazinamium cations, namely the liver and the kidneys. The total amount of radioactivity in the liver was found to be about seven times that found in plasma, whereas in the kidneys it was nine times higher. This supports the theory that quaternary ammonium compounds are excreted by an active mechanism into the bile and it also agrees with our finding that urinary excretion of thiazinamium cations in man is an active process (see Chapter 8 to 11). The amount of radioactivity in urine was about 2.3 times higher than the amount in plasma. It was found that this radioactivity was mainly due to unchanged drug. The ratio between unchanged drug and thiazinamium sulphoxide in urine was 1 : 0.05 which is lower than was found in man after intravenous administration (about 1 : 0.20). On the other hand a substantial amount of sulphoxide was found in the bile. The ratio between unchanged drug and

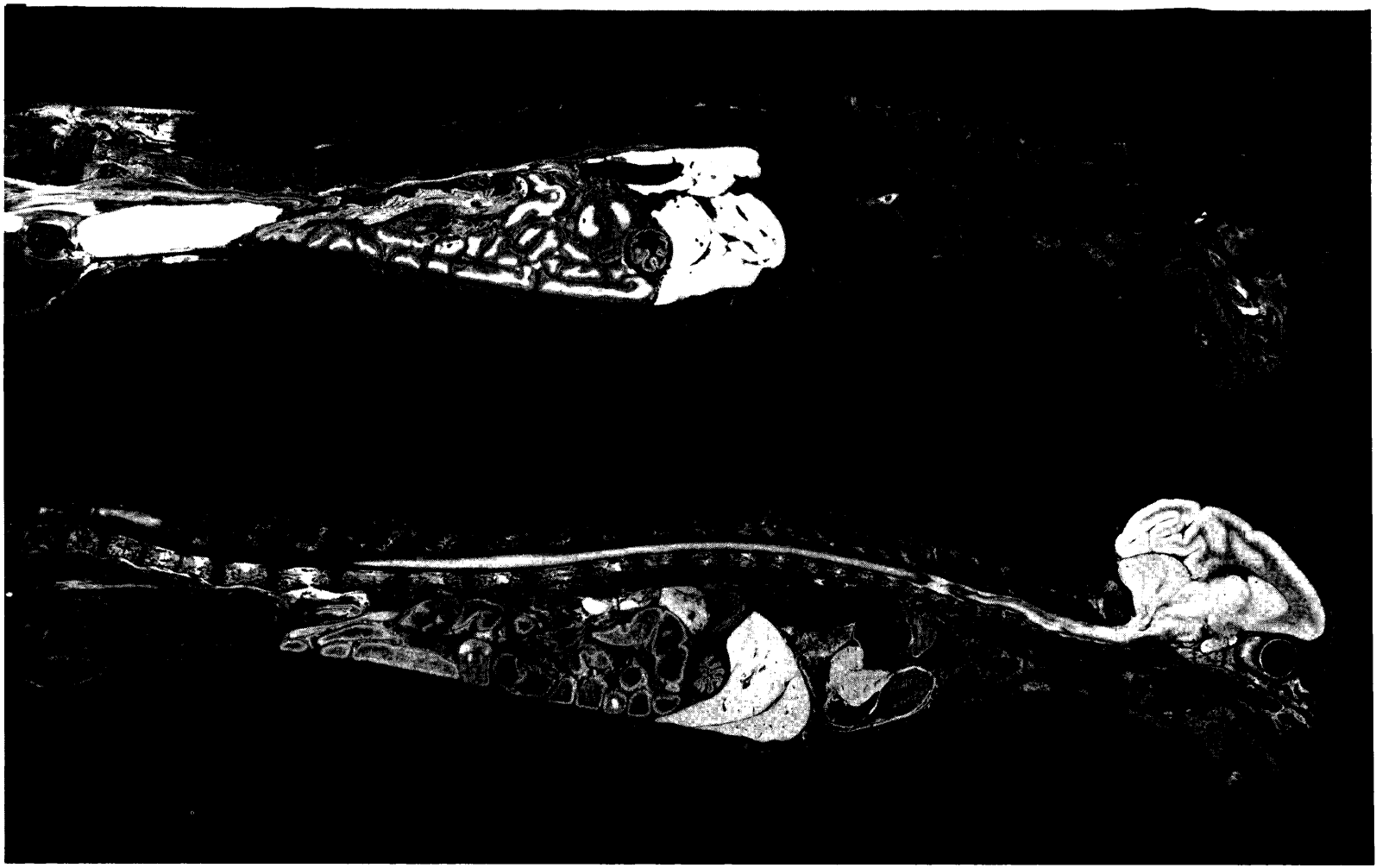


FIG. 2: *Autoradiograms showing the distribution of radioactivity (= white areas) after linear infusion of the quaternary ammonium compound ^{35}S-thiazinamium methylsulphate (above) and its tertiary analogue ^{35}S-promethazine hydrochloride (below) in two squirrel monkeys.*

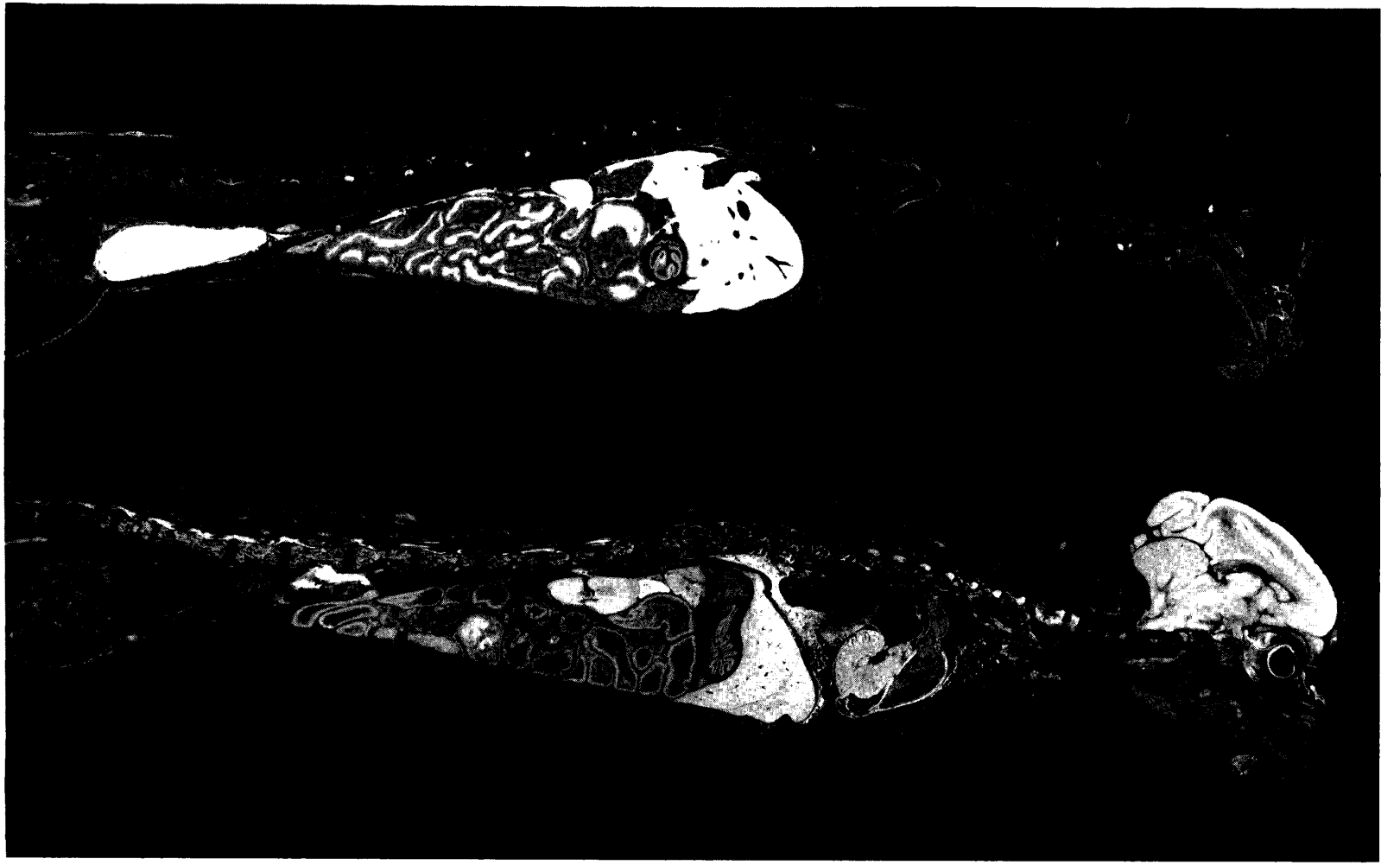


FIG. 3: *Autoradiograms showing the distribution of radioactivity (= white areas) after linear infusion of the quaternary ammonium compound ^{35}S-thiazinamium methylsulphate (above) and its tertiary analogue ^{35}S-promethazine hydrochloride (below) in two squirrel monkeys.*

sulphoxide in the bile of the monkey was 1 : 1, whereas in man a value of 1 : 0.42 was found.
Also, a high level of radioactivity was found in the intestines. Tissue analysis proved that total radioactivity was about ten times that in plasma. The presence in the intestines could be explained as follows.
Knowing that the drug is easily excreted by way of the bile it is possible for it to appear together with the bile in the intestines. Careful analysis of the sections indeed showed that the bile observed in the intestines corresponded with radioactivity. In this way the drug reaches places where absorption could take place, which would lead to enterohepatic cycling. But apart from the presence of radioactivity in the lumen of the intestines it was found that accumulation of radioactivity occurred especially in the mucosa and to a lesser extent in the serosa (see Fig. 4). This may be an indication of the existence of active mechanisms for the transport of thiazinamium cations from the blood into the lumen of the intestines. As already mentioned in Chapter 6, it is assumed that such transport mechanisms exist for other quaternary ammonium ions (LAUTERBACH, 1975). Moreover, this finding agrees with the well known fact that quaternary ammonium ions can easily be adsorbed to the mucin present in the intestines as was already discussed in Chapter 4 (CAVALLITO and O'DELL, 1958; LEVINE and PELIKAN, 1961). So, thiazinamium cations can reach the lumen of the intestines in two ways, namely by biliary excretion and by active secretion through the wall of the intestines.
A considerable amount of radioactivity was also found in gastric mucosa and to a lesser extent in gastric serosa.
High levels of radioactivity were also found in (parts of) organs or tissues with a large number of acetylcholine receptors such as skeletal muscles (*e.g.* myocard), the walls of arteries and especially in the ganglia (see Fig. 3). In the eye a substantial amount of radioactivity was found in the ciliary bodies and a smaller amount in the retina (see Fig. 5a). This agrees with the reported strong anticholinergic activity (see Part I). Accomodation disturbance, sometimes reported as side-effect after a high dose of thiazinamium methylsulphate, can be explained by the high level of the drug in the ciliary bodies. A further accumulation of radioactivity was found in the glandular tissues such as salivary glands, hypophysis, thyroid gland and pancreas. The occurrence of radioactivity in the salivary glands is also in agreement with the clinical observation that salivary secretion is diminished by thiazinamium cations as a result of an anticholinergic effect.
The total amount of thiazinamium cations per gram of tissue of the lung was lower than that found in plasma. But higher concentrations than in plasma were seen in small locations in the lung parenchym.
On the other hand absolutely no drug could be seen on the autoradiograms in the central nervous system (see Fig. 5a). By tissue analysis a very low amount of radioactivity was found, but the concentration in plasma was at

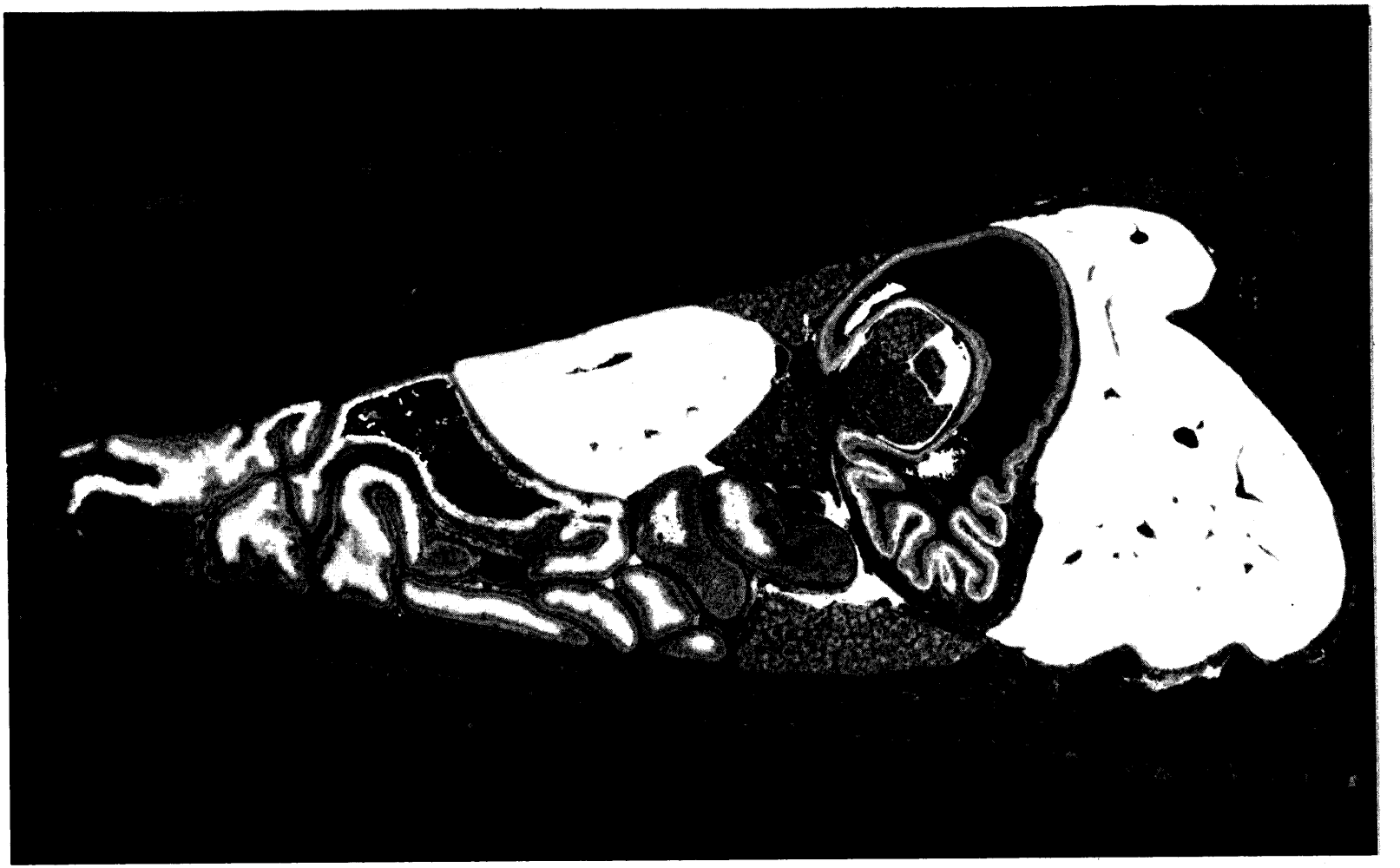


FIG. 4: *Detail of an autoradiogram obtained after linear infusion of ^{35}S-thiazinamium methylsulphate in a squirrel monkey. The autoradiogram shows that high concentrations of radioactivity (= white areas) occur in the mucosa of the intestines and in the gastric mucosa. The concentrations in the serosa are lower.*

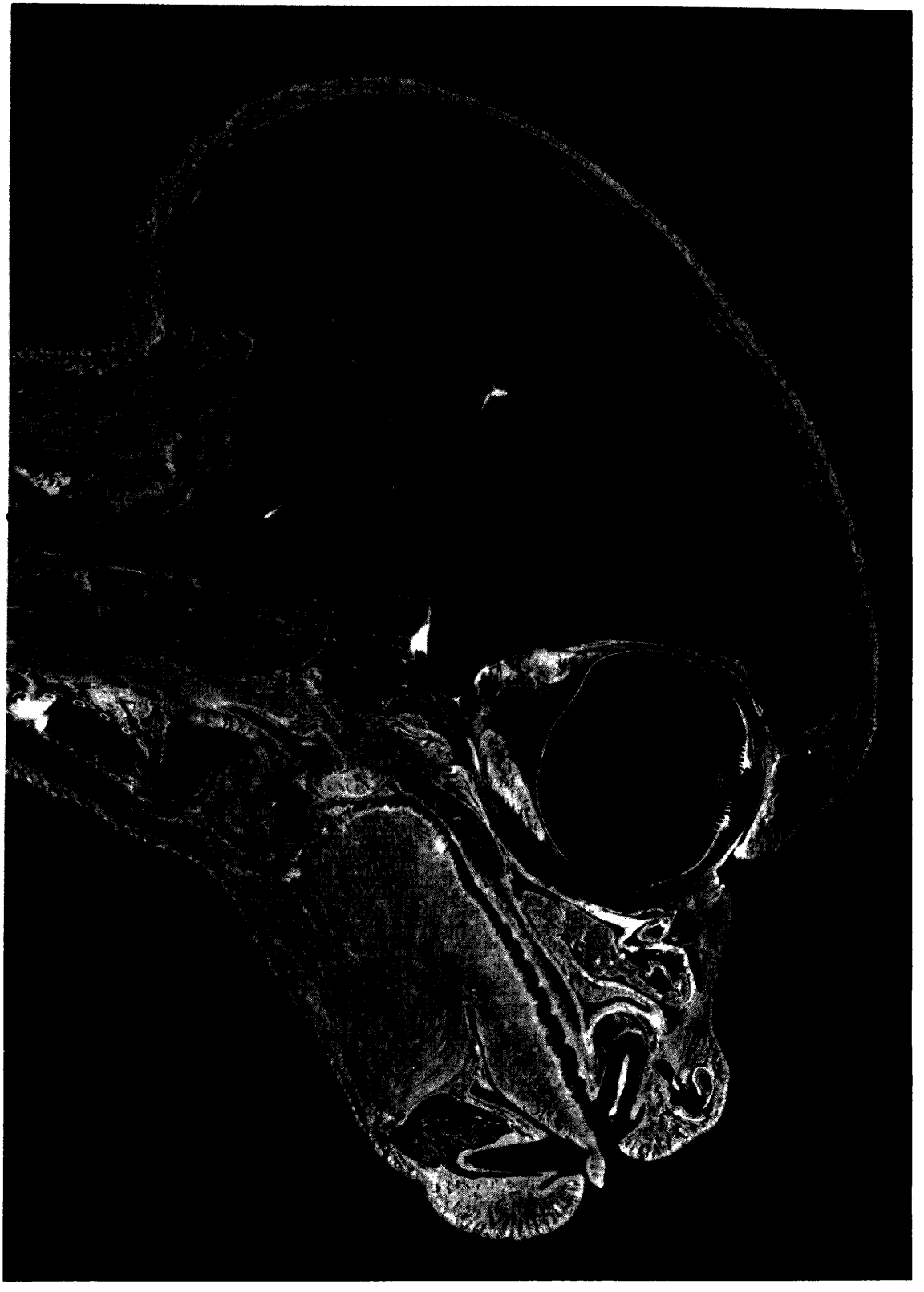


FIG. 5a: *Detail of an autoradiogram obtained after linear infusion of ^{35}S-thiazinamium methylsulphate in a squirrel monkey.*

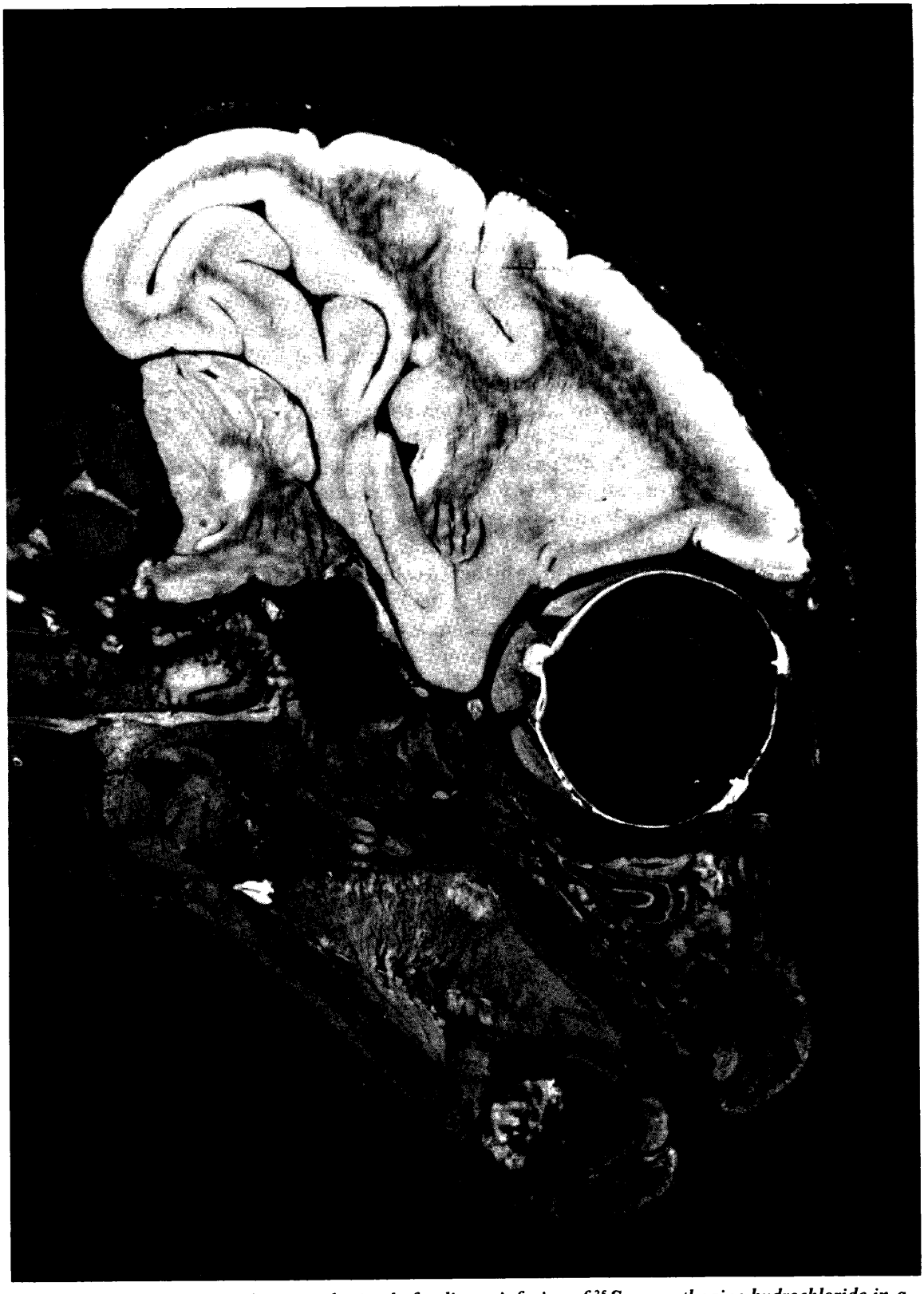


FIG. 5b: *Detail of an autoradiogram obtained after linear infusion of ^{35}S-promethazine hydrochloride in a squirrel monkey.*

least 3000 times higher. Detection of this small amount of radioactivity is hardly significant and may be attributed to the small blood vessels found in this tissue which could not be removed. This results in the counting of radioactivity which is present in the blood in the small vessels. This observation suggests that no significant passage of the "blood-brain barrier" occurs.

From the point of view of medicinal chemistry it is interesting to compare these results with those obtained in an identical study with the tertiary analogue promethazine hydrochloride. Although the pharmacokinetic evaluation of this study has not yet been fully completed, some autoradiograms obtained after infusion in squirrel monkey and also a few of the mice are given here, in order to allow some comparisons.
We will confine ourselves to making some remarks on the significant differences in the distribution of these two drug substances.
The most significant difference with thiazinamium cations is the presence of high levels of radioactivity in the central nervous system indicating that promethazine. HCl can easily pass the "blood-brain barrier". The distribution of the drug in the brain is illustrated in Fig. 5b. Also the amounts of drug in the lungs seemed to be higher as compared to thiazinamium cations. Again accumulations were found in the kidneys but the radioactivity had not (yet) reached the urine bladder, which suggests that in the case of promethazine urinary excretion is not as fast as found for thiazinamium cations. The concentration of radioactivity in the liver was high. Also, considerable radioactivity was observed in the wall of the intestines suggesting that promethazine can also be excreted through the wall of the intestines. As was found for thiazinamium cations, promethazine was present in glandular tissue (salivary glands), the eye and in ganglia which possibly can be explained by the fact that promethazine also has an anticholinergic activity.

13.3.2. Investigations in mice

Generally speaking, the conclusions drawn from the autoradiography study in the squirrel monkey were confirmed after intramuscular injection in mice (see Fig. 6a).
Absorption from the site of injection appeared to start very rapidly, as was also found in the pharmacokinetic studies in man (see Chapter 3). Already two minutes after injection a substantial amount of radioactivity was seen in the liver, the kidneys, the lungs and the mucosa of the intestines, whereas there was no radioactivity in the lumen. After five minutes the concentrations in the liver, kidneys and intestines had increased, whereas in the lung

tissue the drug was now seen in locally high concentrations. This rapid accumulation of thiazinamium cations in liver, kidneys and intestines is probably the main factor determining the fast decline of the plasma concentration-time curve as was found during pharmacokinetic studies in humans (see Chapters 2 and 3).
No radioactivity was observed in the central nervous system.
This distribution pattern remained essentially unchanged during the rest of the experiment. Only two changes could be observed: after 30 min. the radioactivity appeared in the urine bladder and after two hours radioactivity was not only observed in the wall of the intestines but also in the lumen. In the mouse with the longest survival time (six hours) a large amount of radioactivity was still present at the site of injection. The concentrations in the other organs were substantially lower than observed before, except in the urine bladder and the lumen of the intestines. This finding suggests that elimination takes place rapidly, as also was found in man.
The same distribution pattern was observed in pregnant mice. Already 10 min. after the injection a substantial amount of radioactivity was seen in the placenta, but none was found in the foetus. However, two hours after injection a slight concentration of radioactivity was observed in the foetus, especially in its liver. Four hours after the injection the presence of radioactivity in the liver of the foetus could be observed more clearly, whereas the kidneys seemed to contain a rather high amount of radioactivity. Compared to the levels of radioactivity in the placenta the levels in the foetus are low, which suggests that placenta transfer can occur with thiazinamium cations, but that this process only occurs at a low rate and, because of the rapidly systemic elimination, also to a low degree.

It is interesting to compare our results with those reported for two other quaternary phenothiazines, namely Secergan® (ALLGÉN and others, 1960) and Aprobit® (HANSSON and SCHMITTERLÖW, 1961). (See also Chapter 6). In general their conclusions are quite similar to ours. There are only some minor differences, which are possibly caused in part by the difference in survival periods in the experiments. For example for Aprobit® a slow release from the muscle (site of injection) was reported. The first observation was made one hour after injection. However, we found after only two minutes following the injection high levels of radioactivity in liver and kidneys suggesting a fast absorption from the muscle. But after a few hours in both cases a considerable amount of the injected material was still at the site of injection. Furthermore HANSSON and SCHMITTERLÖW reported that after injection of Aprobit® in pregnant mice the placenta contained a large amount of radioactivity but that no radioactivity was seen in the foetuses. We also observed high concentrations in the placenta and also some radioactivity in the foetuses, although to a lesser extent. The differences

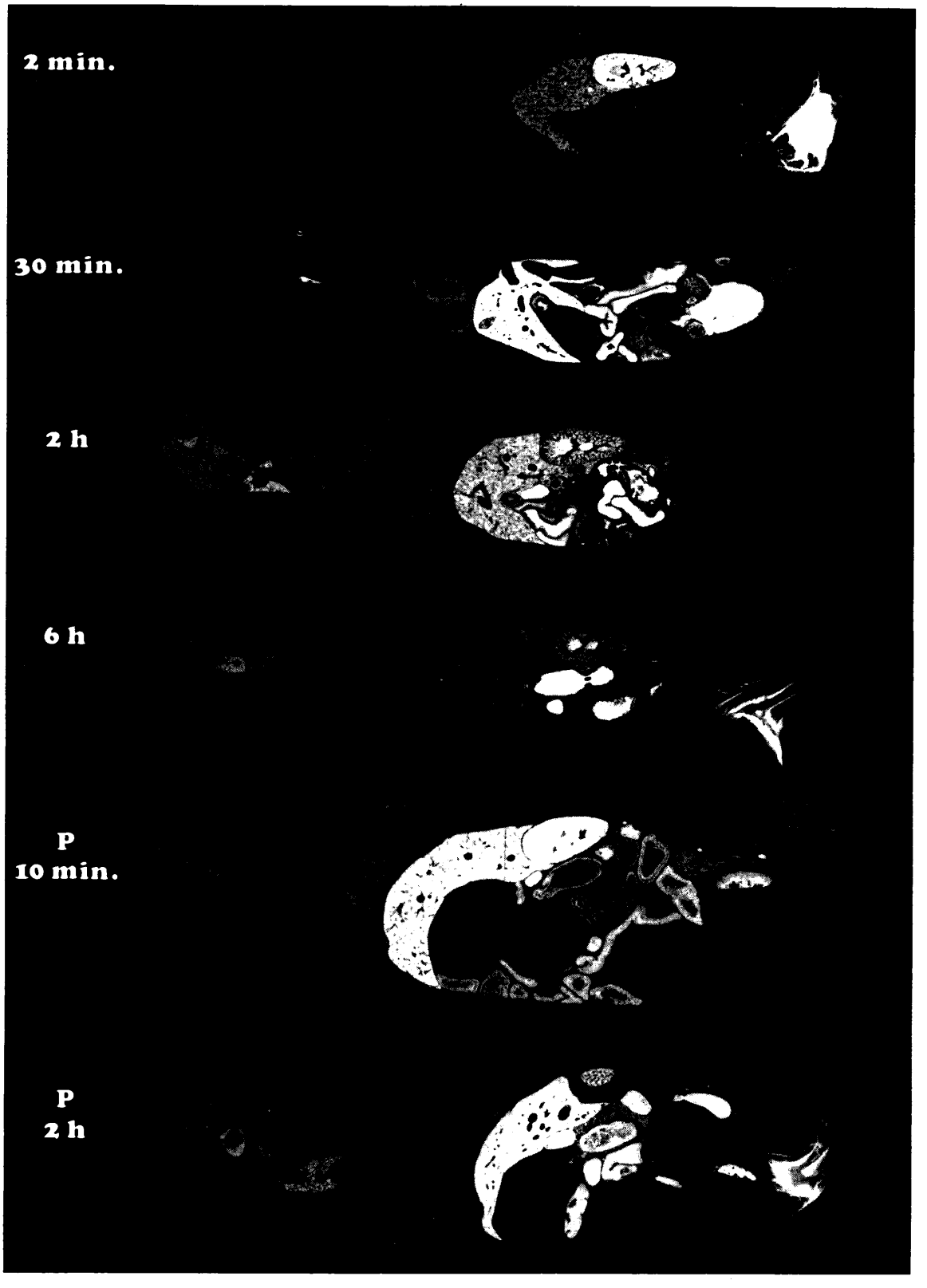


FIG. 6a: *Autoradiograms showing the distribution of radioactivity (= white areas) after intramuscular injection of ^{35}S-thiazinamium methylsulphate in mice and pregnant mice (= P) at various survival periods.*

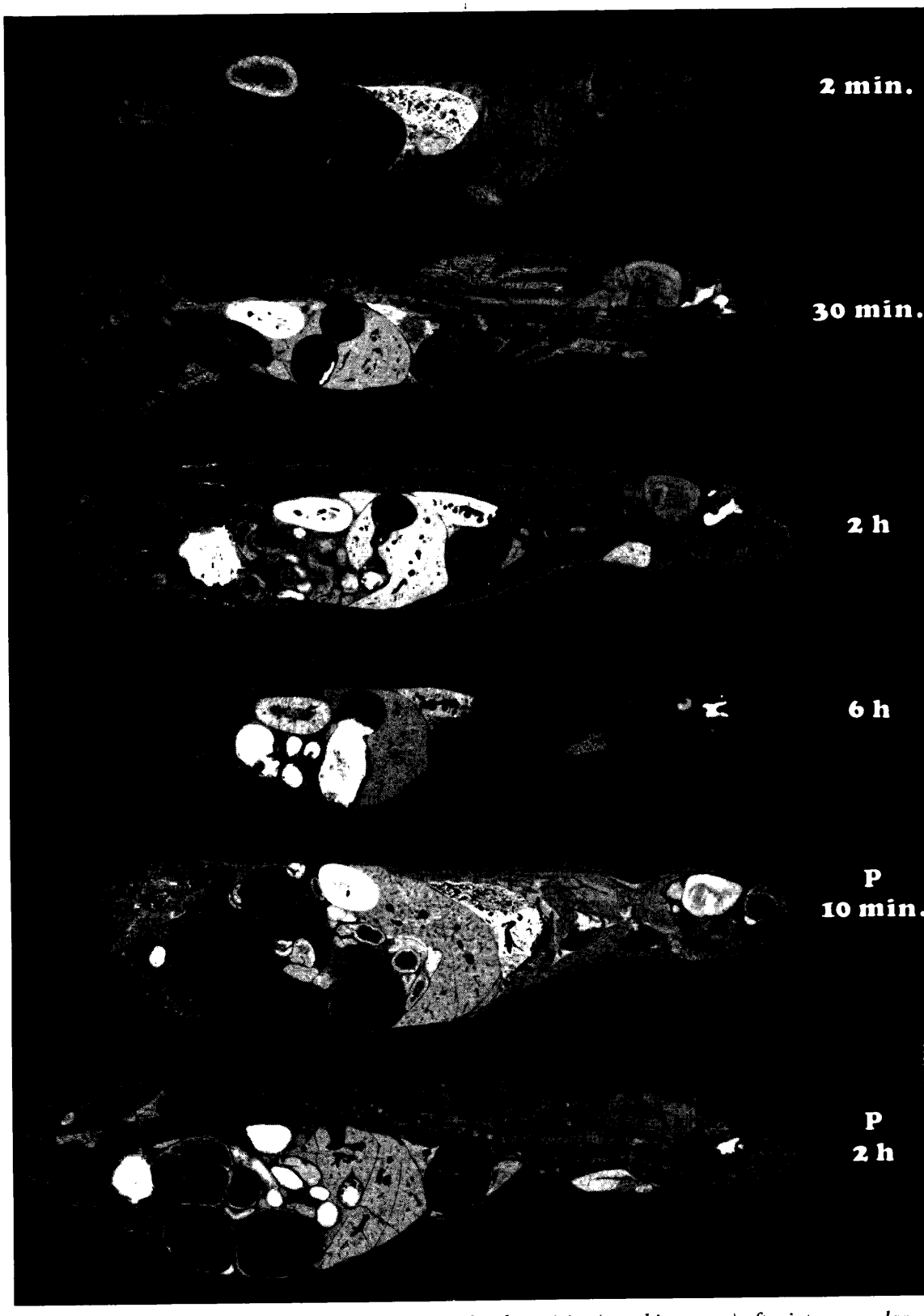


FIG. 6b: *Autoradiograms showing the distribution of radioactivity (= white areas) after intramuscular injection of ^{35}S-promethazine hydrochloride in mice and pregnant mice (=P) at various survival periods.*

observed can not be explained by differences in the doses as these were almost identical.
Secergan® was studied after subcutaneous injection in mice. The distribution pattern of this drug was almost identical of that found for Aprobit®. For Secergan® also no placenta transfer was observed.
Generally speaking the distribution of ^{35}S-thiazinamium methylsulphate is rather similar to that reported for some other quaternary ammonium compounds, not being phenothiazine derivatives, *e.g.* deptropine methiodide (HESPE and PRINS, 1969) and stercuronium iodide (HESPE and WIERIKS, 1971).

Finally we wish to make some observations on promethazine.HCl in mice and pregnant mice (see Fig. 6b). In general terms the distribution pattern of promethazine.HCl in mice is identical to that found in monkey, as was discussed above.
Comparison of the distribution of thiazinamium cations and promethazine in pregnant mice led us to the conclusion that for promethazine, placenta transfer can occur more easily and distribution of the latter drug in the foetus was not restricted to the liver and kidneys as happened with thiazinamium cations.

Conclusion

After linear infusion of ^{35}S-thiazinamium methylsulphate in a squirrel monkey accumulation of radioactivity was found in the liver (and gall bladder), the kidneys (and urine bladder) and intestines, being the three main pathways of excretion for thiazinamium cations.
A considerable concentration of radioactivity was also observed in organs with high amounts of acetylcholine receptors such as the ganglia, skeletal muscles (and myocard) and the ciliary bodies in the eye.
Glandular tissue (salivary glands, hypophysis, thyroid gland and pancreas) contained high levels of radioactivity.
On the other hand, no thiazinamium cations could be found in the central nervous system, which suggests that the quaternary ammonium ion cannot pass the "blood-brain barrier".
After intramuscular injection of the drug in mice it was found that absorption starts rapidly, but six hours after injection a considerable residual amount of drug was still present at the site of injection.
In pregnant mice high concentrations of radioactivity were observed in the placenta, but only low amounts were found in the liver and the kidneys of the foetuses. This implies that with thiazinamium cations placenta transfer can occur, although at a low rate and to a less extent.
The results of an identical study with the tertiary analogue, promethazine hydrochloride, were discussed in short terms.

References

ALLGÉN, L. G., L. EKMAN, L. REIO and S. ULLBERG (1960), *Arch. Int. Pharmacodyn. 126,* 1

CAVALLITO, C. J. and TH. B. O'DELL (1958), *J. Am. Pharm. Assoc. Sc. Ed. 47,* 169

HANSSON, E. and C. G. SCHMITTERLÖW (1961), *Arch. Int. Pharmacodyn. 131,* 309

HESPE, W. and H. PRINS (1969), *Biochem. Pharmacol. 18,* 53

HESPE, W. and J. WIERIKS (1971), *Biochem. Pharmacol. 20,* 1213

KLEIJN, E. VAN DER (1969), *"Pharmacokinetics of Ataractic Drugs"*, thesis, Nijmegen, The Netherlands

LAUTERBACH, F. (1975), *Arzneimittel-Forsch. 25,* 479

LEVINE, R. R. and E. W. PELIKAN (1961), *J. Pharmacol. Exp. Ther. 131,* 319

ULLBERG, S. (1954), *Acta Radiol. Suppl.,* 118

ULLBERG, S. (1958), *Proceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy, 24,* 248

Summary

This thesis presents the results of some investigations concerning the bioanalysis and pharmacokinetics (including biotransformation) of thiazinamium methylsulphate (Multergan®). The drug is a phenothiazine derivative with a quaternary ammonium group in the molecule. Because of its antihistaminic and anticholinergic properties, the drug is in use for patients suffering from generalized obstructive lung diseases.

In **Part I,** called "A profile of the drug under study", some analytical chemical, physico-chemical and pharmacological data of thiazinamium methylsulphate are dealt with, which are related to its bioanalysis and pharmacokinetics.

Part II is entitled "Bioanalysis".
In *Chapter 1* the general principles and a few theoretical aspects of the ion pair extraction method are discussed. Various applications and possibilities are mentioned and a review based on the literature is given, of the analysis of about 100 drugs using this method. Special attention is paid to the usefulness of the method for the isolation of drugs and drug metabolites in body fluids.
In *Chapter 2* the development of a sensitive and selective method for the quantitative determination of thiazinamium cations in plasma, urine, bile and saliva is described. The procedure is based on ion pair extraction of the quaternary ammonium compound with iodide as counter ion. Optimum extraction conditions were established on the basis of the extraction constant and the constant for side reaction. The ion pair extraction was followed by gas chromatography with alkali flame ionization detection ("nitrogen detector"). Thiazinamium sulphoxide cations, which was found to be the only metabolite in humans, do not interfere in the present procedure. Its sensitivity allows the determination of plasma concentrations as they are obtained following the administration of therapeutic doses of the drug.
In *Chapter 3* the development of a method for the isolation from urine and bile and quantitative determination of thiazinamium sulphoxide cation is described. The isolation method was based on column chromatography with Amberlite XAD-2®, followed by two-dimensional thin-layer chroma-

tography. The spots were visualized by immersion in an oxidative mixture and quantitation was done by measuring the transmission of the spots with a "flying spot" densitometer.

In **Part III,** called "Pharmacokinetics", the biopharmaceutical and pharmacokinetic studies, including biotransformation and excretion are described. *Chapter I* contains some general remarks on bioavailability. Definitions of the absolute and the relative bioavailability are given and some methods for determining them are described.
In *Chapter 2* the pharmacokinetics of thiazinamium cations after intravenous injection in humans are discussed. It appeared that the data observed fitted well into a two-compartment open model. Pharmacokinetic parameters were calculated using the NAFFIT-1 and NONLIN programmes and the results obtained with both programmes were compared. A short α-phase (distribution phase) with a mean half-life of about 6 min. was found. The apparent volume of distribution of the central compartment was approximately 20 l. The β-phase (elimination phase) had a much longer half-life (approximately 300 to 600 min.) and the apparent volume of distribution of the peripheral compartment was to the order of 200 to 300 l. A large interindividual variation was found, particularly in the data of the β-phase.
In *Chapter 3* the pharmacokinetics following intramuscular injection are discussed. The absolute bioavailability during the time of the experiment was 100%. The absorption from the muscle was very fast. Peak plasma concentrations were generally seen at about 10 min. after the injection was given. Injection technique, the injection site and the capillary blood flow seemed to be important factors in relation to the final profile of the plasma concentration-time curve. The value for the half-life in the α-phase was approximately 20 min. and the apparent volume of distribution for the central compartment was 40 to 60 l. During the β-phase the half-life was 300 to 600 min. and the apparent volume of distribution was 200 to 400 l.
Chapter 4 deals with the bioavailability of the drug after oral administration. Thiazinamium cations, in spite of the relatively high molecular weight and the presence of a quaternary ammonium group, can be absorbed from the gastrointestinal tract. Although the bioavailability was low (to the order of 10%), the plasma concentration-time curve showed a long-lasting plateau value which ensured therapeutic plasma concentration for at least three to six hours, provided that a sufficiently large dose is given. At the end of the observation period (seven hours) the plasma concentrations had not yet fallen to a zero concentration. A substantial interindividual variation and a lower intraindividual variation in the bioavailability was reported.
In *Chapter 5* the bioavailability observed after rectal administration is discussed. Two suppository bases were investigated, namely Witepsol H-15 and a polyethylene glycol mixture (1500/4000 in a ratio of 1 : 2). It was

shown, that thiazinamium cations could be absorbed from the human rectum. The best results were obtained with Witepsol H-15, with which the relative bioavailability was of the same order of magnitude as after oral administration, but the peak plasma concentrations appeared earlier in the case of rectal administration (after about 60 min.) and in general the curve had already declined to zero within seven hours. Interindividual variation was also similar to that obtained after oral administration. After the application of the drug in the polyethylene glycol base, very low plasma concentrations were found and the bioavailability was almost negligible. Attempts to increase the absorption of thiazinamium by applying the drug as an ion pair were unsuccessful: the absorption of thiazinamium iodide and thiazinamium salicylate was even poorer or only as good as that of the methylsulphate.

In *Chapter 6* some general remarks are made on the biotransformation and excretion of quaternary ammonium compounds, especially those also containing a phenothiazine group.

Chapter 7 deals with the results of a qualitative study on biotransformation and excretion of thiazinamium cations. Thiazinamium sulphoxide was found to be the only metabolite in humans. Neither ring-hydroxylation products nor demethylation products (*e.g.* promethazine) could be detected. Thiazinamium sulphoxide was found both in urine and bile.

In the successive chapters the rate and the extent of the excretion of the unchanged drug and the sulphoxide are discussed as obtained following different routes of administration.

In *Chapter 8* it is reported that after an intravenous injection about 41.4% of the dose was excreted, unchanged, in the urine. The excretion was very rapid and almost complete within eight hours. About 9.2% of the dose was excreted in the urine in the form of thiazinamium sulphoxide cations, which gave a mean ratio between unchanged drug and metabolite of 1 : 0.22.

Chapter 9 describes the excretion of thiazinamium cations and thiazinamium sulphoxide cations after giving the drug by an intramuscular injection. Three different doses were used. After a dose of 6.25 mg or 12.5 mg the excretion was essentially complete within eight hours, but this was not the case after the administration of a dose of 25.0 mg. No correlation could be found between the urine production or pH and the amount of drug excreted in urine. About 40% of the dose was excreted unchanged and about 10% of the dose in the form of the sulphoxide. The ratio between the amounts of unchanged drug and metabolite was a rather constant value in each patient, but substantial interindividual variations were observed. In a study involving bile-fistula patients it was found that both thiazinamium cations and thiazinamium sulphoxide cations are excreted to a considerable extent in bile. The amount of unchanged drug was almost equal to that in the urine but the amount of sulphoxide was slightly higher (ratio about 1 : 0.42).

In *Chapter 10* it is shown that the biotransformation and excretion pattern after oral administration are considerably different from those after parenteral administration. The individual renal clearance values were calculated. The mean value was 256 ± 136(S.D.) ml/min., which is considerably higher than the value for glomerular filtration, based on creatinine clearance. This is an indication of the occurrence of an active excretion process (tubular secretion). Hepatic clearance (biotransformation and biliary excretion of unchanged drug) accounted for 537 ± 495(S.D.) ml/min. There existed a correlation between the value of hepatic clearance and the amount of sulphoxide in urine, whereas the hepatic clearance and bioavailability were negatively correlated. The ratio between the unchanged drug and the metabolite in urine was now 1 : 0.94 which was substantially different from that found after intramuscular injection in the same patients (1 : 0.20). This is an indication of a "first pass effect". It was calculated that about half of the absorbed amount of thiazinamium cations was metabolized during the first liver passage.

The fate of thiazinamium cations after rectal administration is described in *Chapter 11*, where particular attention was paid to suppositories with Witepsol H-15 as base. Within the first seven hours of the study, about 85% of the totally excreted amount of unchanged drug was found in the urine. Within 24 hours the excretion was complete and about 38.8% of the dose was excreted in the unchanged form whereas about 30.4% was excreted as sulphoxide. This gave a mean ratio between unchanged drug and sulphoxide in the urine of 1 : 0.78 which was substantially different from that after intramuscular injection in the same individuals (about 1 : 0.24). This difference has been explained by assuming a "first pass effect". It was calculated that about 34.5% of the absorbed amount of drug was subjected to biotransformation during the first liver passage, which was of the same order of magnitude as found after oral administration. This led to the conclusion that the "first pass effect" of thiazinamium cations can not be avoided by giving the drug rectally.

In *Chapter 12* some results are reported on the correlation between the plasma concentration of thiazinamium cations and the heart rate. Special attention was paid to the question of how far monitoring of the heart rate can be of help in determining the bioavailability of thiazinamium methylsulphate after oral administration for example. Some correlation between the plasma concentration and the heart rate was found. This correlation was better when dealing with higher plasma concentrations and accordingly greater changes in the heart rate, and was possibly due to inaccuracy of the method of monitoring the heart rate. However, it appeared to be possible to obtain a rough impression of the order of magnitude of the relative bioavailability of the drug after oral administration by calculating it from the areas under the heart rate-time curves, obtained after oral and intramuscular administration.

Finally in *Chapter 13* the distribution of ^{35}S-ringlabelled thiazinamium methylsulphate has been studied by means of whole body autoradiography in a squirrel monkey and in mice. In the monkey, in "steady state", high accumulations of radioactivity were found in the three main organs of excretion of the drug (liver, kidneys and intestines). High concentrations of radioactivity were also observed in organs with high amounts of acetylcholine receptors such as the ganglia, skeletal muscles (myocard) and ciliary bodies, and in the glandular tissue (salivary glands, hypophysis, thyroid gland and pancreas). On the other hand no radioactivity was seen in the central nervous system, suggesting that thiazinamium cations cannot pass the "blood-brain barrier". After intramuscular injection of the drug in mice, it was found that the absorption started rapidly, but six hours after the injection, a considerable residual amount was still present at the site of injection. In pregnant mice high concentrations of radioactivity were observed in the placenta, but only low amounts were found in the liver and the kidneys of the foetuses, which implies that with thiazinamium cations, placenta transfer can occur, although to a low extent and at a low rate. The results of an identical study with the tertiary analogue, ^{35}S-promethazine hydrochloride, were discussed briefly.

Samenvatting

In dit proefschrift worden de resultaten beschreven van een onderzoek betreffende de bioanalyse en farmacokinetiek (met inbegrip van de biotransformatie) van thiazinamium methylsulfaat (Multergan®). Thiazinamium methylsulfaat is een fenothiazine-derivaat en heeft een kwaternaire ammonium groep in de alifatische zijketen. Als gevolg hiervan bezit het zowel antihistamine als anticholinerge activiteit. Het geneesmiddel is in gebruik bij bepaalde vormen van CARA (Chronische Aspecifieke Respiratoire Aandoeningen).

In **Deel I** wordt een aantal analytisch-chemische, fysisch-chemische en farmacologische eigenschappen van thiazinamium methylsulfaat besproken, vooral voor zover ze gerelateerd zijn aan de bioanalyse en de farmacokinetiek van dit farmacon.

Deel II heeft bioanalyse als onderwerp.
In *Hoofdstuk 1* worden de algemene principes en enkele theoretische aspecten van de ionpaar-extractie methode besproken. Verschillende toepassingen en mogelijkheden worden genoemd en er wordt een literatuuroverzicht gegeven van meer dan 100 toepassingen van ionpaar-extractie. In het bijzonder wordt aandacht besteed aan de bruikbaarheid van de methode voor isolatie van geneesmiddelen en hun metabolieten uit lichaamsvloeistoffen.
In *Hoofdstuk 2* wordt de ontwikkeling van een gevoelige en selectieve methode voor de kwantitatieve bepaling van thiazinamium kationen in plasma, urine en gal besproken. De bepalingsmethode is gebaseerd op ionpaar-extractie van het kwaternaire ammonium ion met jodide als tegen-ion. De optimale omstandigheden voor de extractie werden bepaald op basis van de extractie-constante en de constante voor een nevenreactie. De ionpaar-extractie werd gevolgd door een gaschromatografische bepaling met alkali vlam ionisatie detectie (een z.g.n. "stikstof-detector"). De enige metaboliet die bij de mens is aangetoond, te weten het thiazinamium sulfoxide kation, stoorde niet. De gevoeligheid was voldoende om plasmaconcentraties, verkregen na toediening van een therapeutische dosis van het geneesmiddel, adequaat te bepalen.
In *Hoofdstuk 3* is de ontwikkeling van een methode beschreven voor de

isolatie - uit urine en gal - en kwantitatieve bepaling van de thiazinamium sulfoxide kationen. De isolatie was gebaseerd op kolomchromatografie met Amberlite XAD-2® als kolomvulling. Het eluaat werd na concentreren onderworpen aan een twee-dimensionale dunnelaagchromatografische scheiding. De vlekken van het sulfoxide werden zichtbaar gemaakt door onderdompeling in een oxiderend mengsel en kwantitatieve bepaling vond plaats door middel van een *"in situ"* meting van de transmissie met behulp van een "flying spot" densitometer.

In **Deel III** worden de biofarmaceutische en farmacokinetische onderzoekingen (met inbegrip van de biotransformatie en de uitscheiding) besproken.

Hoofdstuk 1 bevat een aantal algemene opmerkingen over het begrip biologische beschikbaarheid. De definities van de absolute en de relatieve biologische beschikbaarheid worden gegeven en methoden voor de bepaling ervan worden beschreven.

In *Hoofdstuk 2* wordt de farmacokinetiek van thiazinamium kationen, na intraveneuze injectie in de mens beschreven. Er bleek sprake te zijn van een open twee-compartimenten model. Diverse farmacokinetische parameters werden berekend met behulp van het NAFFIT-1 en het NONLIN programma. De resultaten verkregen met deze twee programma's werden met elkaar vergeleken. Er werd een korte α-fase (distributie-fase) gevonden, met een halfwaarde-tijd van ongeveer 6 min. Het schijnbare verdelingsvolume van het centrale compartiment was ongeveer 20 l. De β-fase (eliminatie-fase) bleek een veel langere halfwaarde-tijd, n.l. in de orde van grootte van 300 tot 600 min., te hebben. Het schijnbare verdelingsvolume van het perifere compartiment was in de orde van grootte van 200 tot 300 l. Er werd een grote interindividuele variatie gevonden, vooral in de waarden van de farmacokinetische parameters die betrekking hebben op de β-fase.

In *Hoofdstuk 3* wordt de farmacokinetiek na intramusculaire injectie besproken. De absolute biologische beschikbaarheid gedurende de waarnemingsperiode (zeven uren) was 100%. De resorptie vanuit het spierweefsel was snel. Ongeveer 10 min. na de injectie werd het maximum in de plasmaconcentratie-tijd curve bereikt. De injectie-techniek, de plaats van injectie en de capillaire doorbloeding bleken van grote invloed op de uiteindelijke vorm van de plasmaconcentratie-tijd curve. De halfwaarde-tijd in de α-fase was ongeveer 20 min. en het schijnbare verdelingsvolume voor het centrale compartiment was 40 tot 60 l. Gedurende de β-fase was de halfwaarde-tijd ongeveer 300 tot 600 min. en het schijnbare verdelingsvolume voor het perifere compartiment was ongeveer 200 tot 400 l.

In *Hoofdstuk 4* wordt aandacht besteed aan de biologische beschikbaarheid na orale toediening. Het bleek dat thiazinamium kationen, ondanks het vrij hoge molecuulgewicht en ondanks de aanwezigheid van een kwaternaire

ammonium groep, wel degelijk geresorbeerd kunnen worden na orale toediening. Weliswaar was de biologische beschikbaarheid laag (in de orde van grootte van 10%), maar de plasmaconcentratie-tijd curve vertoonde een plateauvormig verloop dat ongeveer drie tot zes uren aanhield. Aan het eind van de waarnemingsperiode (zeven uren) was de plasmaconcentratie-tijd curve in de meeste gevallen nog niet tot nul gedaald. Dit betekent, dat, mits voldoende hoog gedoseerd is, gedurende vrij lange tijd plasmaconcentraties boven een therapeutisch minimum verkregen kunnen worden. Er werd een vrij aanzienlijke interindividuele variatie en een lagere intraindividuele variatie in de biologische beschikbaarheid gevonden.

In *Hoofdstuk 5* worden de resultaten, verkregen na rectale toediening, besproken. Twee zetpil-bases werden in het onderzoek betrokken, namelijk Witepsol H-15 en een polyethyleenglycol mengsel (1500/4000 in een verhouding van 1 : 2). Het bleek, dat thiazinamium kationen na rectale toediening geresorbeerd kunnen worden. De beste resultaten werden verkregen met Witepsol H-15, waarmee een relatieve biologische beschikbaarheid werd bereikt, die in dezelfde orde van grootte lag als die welke was gevonden na orale toediening. Het maximum in de plasmaconcentratie-tijd curve verscheen na rectale toediening eerder (na ongeveer 60 min.) dan in het geval van orale toediening. Het resorptie proces na rectale toediening duurde korter dan na orale toediening, want na zeven uren was de plasmaconcentratie in de meeste gevallen reeds tot nul gedaald. Na toediening van thiazinamium methylsulfaat in de polyethyleenglycol basis werden zeer lage plasmaconcentraties gevonden en was de biologische beschikbaarheid vrijwel verwaarloosbaar. Pogingen om de resorptie van thiazinamium te vergroten door toediening in de vorm van een ionpaar hadden weinig succes: resorptie van thiazinamium jodide en thiazinamium salicylaat was gelijk aan de resorptie van het methylsulfaat of zelfs slechter.

In *Hoofdstuk 6* wordt een literatuur-overzicht gegeven van de biotransformatie en excretie van kwaternaire ammonium verbindingen, die tevens een fenothiazine groep in het molecuul bevatten.

In *Hoofdstuk 7* worden de resultaten behandeld van een kwalitatieve studie betreffende biotransformatie en excretie van thiazinamium kationen. Bij de mens werd slechts één metaboliet - thiazinamium sulfoxide - gevonden. Er konden géén ring-hydroxylerings- of demethyleringsprodukten (b.v. promethazine) worden aangetoond. Het thiazinamium sulfoxide werd zowel in de urine als in de gal gevonden.

In de daarop volgende hoofdstukken wordt de mate van uitscheiding en de uitscheidingssnelheid van het onveranderde geneesmiddel en het sulfoxide beschreven, zoals deze gevonden werden na verschillende wijzen van toediening.

In *Hoofdstuk 8* wordt meegedeeld, dat na intraveneuze injectie ongeveer 41.4% van de dosis onveranderd in de urine werd uitgescheiden. de uitscheiding ervan was snel en bijna compleet binnen acht uren na injectie.

Ongeveer 9.2% van de dosis werd in de urine uitgescheiden in de vorm van thiazinamium sulfoxide kationen, hetgeen resulteerde in een gemiddelde verhouding tussen onveranderd geneesmiddel en metaboliet van 1 : 0.22.
In *Hoofdstuk 9* wordt nader ingegaan op de uitscheiding van thiazinamium kationen en thiazinamium sulfoxide kationen na intramusculaire injectie van het geneesmiddel. Drie verschillende doses werden gebruikt. Na injectie van een dosis van 6.25 of 12.5 mg was de excretie vrijwel compleet binnen acht uren, maar dit was niet het geval na een injectie van 25.0 mg. Er kon geen correlatie worden aangetoond tussen de urine-produktie of de pH en de hoeveelheden onveranderd geneesmiddel en metaboliet in de urine. Ook na intramusculaire injectie werd ongeveer 40% van de dosis als thiazinamium kationen en 10% van de dosis als thiazinamium sulfoxide kationen uitgescheiden. De verhouding tussen de hoeveelheden van het onveranderde geneesmiddel en de metaboliet in de urine bleek voor iedere patient een vrij constante waarde te hebben, maar daarentegen werden grote interindividuele verschillen waargenomen. In een onderzoek in patienten met een gal-fistel werd gevonden, dat zowel thiazinamium kationen als thiazinamium sulfoxide kationen in de gal worden uitgescheiden. De hoeveelheid van het onveranderde geneesmiddel in de gal was ongeveer gelijk aan de hoeveelheid die in de urine werd gevonden, maar de hoeveelheid van het sulfoxide was hoger in de gal (verhouding ongeveer 1 : 0.42).
In *Hoofdstuk 10* wordt aangetoond, dat de biotransformatie en het uitscheidings-patroon na orale toediening van thiazinamium methylsulfaat geheel verschillend zijn van die na parenterale toediening. De waarden voor de renale klaring werden uitgerekend. De gemiddelde waarde was 256 ± 136 (S.D.) ml/min., hetgeen aanzienlijk hoger is dan de glomerulaire filtratie, gebaseerd op de creatinine klaring. Dit vormt een aanwijzing voor het bestaan van een actief uitscheidingsproces (tubulaire secretie). De lever-klaring (biotransformatie en uitscheiding via de gal van het onveranderde geneesmiddel) bedroeg 537 ± 495 (S.D.) ml/min. Er bleek een correlatie te bestaan tussen de waarde van de lever-klaring en de hoeveelheid van het sulfoxide in de urine, terwijl de lever-klaring en de biologische beschikbaarheid negatief waren gecorreleerd. De verhouding tussen het onveranderde geneesmiddel en de metaboliet in urine was na orale toediening 1 : 0.94, hetgeen aanzienlijk verschilt van de verhouding na intramusculaire toediening in dezelfde patienten (1 : 0.20). Dit is een indicatie voor het bestaan van een "first pass effect". Uit berekeningen bleek, dat ongeveer de helft van de geresorbeerde thiazinamium kationen werd gebiotransformeerd tijdens de eerste passage door de lever.
Biotransformatie en uitscheiding na rectale toediening van thiazinamium methylsulfaat is beschreven in *Hoofdstuk 11*. In het bijzonder werd aandacht besteed aan zetpillen met Witepsol H-15 als basis. De uitscheiding was snel. Binnen zeven uren werd ongeveer 85% van de totaal uitgescheiden

hoeveelheid van het onveranderde geneesmiddel in de urine gevonden. In 24 uur was de uitscheiding compleet en werd ongeveer 38.8% van de dosis in onveranderde vorm uitgescheiden, terwijl 30.4% werd uitgescheiden in de vorm van het sulfoxide. Dit resulteerde in een verhouding in de urine van 1 : 0.78, hetgeen aanzienlijk verschilt van de verhouding, die gevonden werd na intramusculaire toediening in dezelfde patienten (ongeveer 1 : 0.24). Dit verschil werd verklaard door aan te nemen dat er sprake was van een aanzienlijk "first pass effect". Er werd berekend, dat ongeveer 34.5% van de geresorbeerde hoeveelheid van de thiazinamium kationen tijdens de eerste lever-passage werd gebiotransformeerd, hetgeen vergelijkbaar is met de waarnemingen na orale toediening. Een en ander leidde tot de conclusie, dat "first pass effect" van thiazinamium kationen niet vermeden kon worden door het geneesmiddel rectaal toe te dienen.

In *Hoofdstuk 12* wordt kort melding gemaakt van het bestaan van een zekere correlatie tussen de plasmaconcentratie van de thiazinamium kationen en de hartslag. In het bijzonder werd aandacht besteed aan de vraag in hoeverre het mogelijk is de relatieve biologische beschikbaarheid van thiazinamium methylsulfaat (na b.v. orale toediening) te bepalen door registratie van de hartslag. De correlatie, die er bleek te bestaan, is niet in alle gevallen erg goed, b.v. wanneer sprake is van lage plasmaconcentraties en kleine veranderingen in de hartslag. Dit is waarschijnlijk te wijten aan de onnauwkeurigheid van de hartslagmeting. De correlatie was duidelijk beter wanneer sprake was van vrij grote veranderingen in de hartslag. Het bleek evenwel mogelijk om een globale indruk te krijgen van de orde van grootte van de relatieve biologische beschikbaarheid van het geneesmiddel na orale toediening, door berekening uit de oppervlakten onder de hartslag-tijd curves die verkregen zijn na orale en intramusculaire toediening.

Tenslotte worden in *Hoofdstuk 13* de resultaten besproken van een onderzoek naar de verdeling van ^{35}S-gemerkt thiazinamium methylsulfaat. Dit onderzoek werd uitgevoerd door middel van autoradiografie in een eekhoorn-aapje en in muizen. Na lineaire infusie tot "steady state" concentratie in de aap, werd een sterke ophoping van radioactiviteit gevonden in de drie uitscheidingsorganen voor thiazinamium kationen, te weten de lever, de nieren en de darmen. Hoge concentraties radioactiviteit werden ook gevonden in organen met veel acetylcholine receptoren, zoals de ganglia, dwarsgestreepte spieren (myocard) en corpus ciliare alsmede in endocriene klieren (speekselklieren, hypophyse, schildklier en alvleesklier). Anderzijds werd geen radioactiviteit waargenomen in het centrale zenuwstelsel, hetgeen suggereert dat thiazinamium kationen de "bloed-hersen barrière" niet kunnen passeren. Na intramusculaire injectie van het geneesmiddel in muizen bleek, dat de resorptie uit de spier zeer snel op gang komt, maar zes uren na de injectie was nog een aanzienlijke hoeveelheid radioactiviteit aanwezig op de plaats van de injectie. In zwangere muizen

werden hoge concentraties radioactiviteit waargenomen in de placenta, maar slechts geringe hoeveelheden werden aangetroffen in de lever en de nieren van de vruchten. Dit houdt in dat thiazinamium kationen de placenta kunnen passeren, maar kennelijk met een lage snelheid en in beperkte mate. De resultaten van een identiek onderzoek met ^{35}S-gemerkt promethazine hydrochloride - de tertiaire analoog - worden kort besproken.

Curriculum vitae

Jan Hasker Gerhardus Jonkman werd geboren op 15 februari 1946 te Oosterwolde (Fr.). Hij bezocht van 1958–1963 de Rijks Hogere Burgerschool te Oosterwolde (Fr.) (diploma H.B.S.-B). Vervolgens studeerde hij farmacie aan de Rijksuniversiteit te Groningen. Tijdens het bijvak "Dierfysiologie" werd de basis gelegd voor zijn belangstelling voor de farmacokinetiek in het algemeen en in het bijzonder voor de wijze waarop deze werd beïnvloed door de toedieningswijze en de vormgeving van het geneesmiddel. Het doctoraalexamen werd in 1970 afgelegd. Het apothekersexamen vond in 1971 plaats.
Sindsdien is hij werkzaam op het Laboratorium voor Farmaceutische en Analytische Chemie der Rijksuniversiteit te Groningen. Daar herschreef hij samen met Prof. Dr. J. S. Faber het collegedictaat farmaceutische chemie, getiteld "Geneesmiddelen met werking op het zenuwstelsel". Verder leidde hij als hoofdassistent enkele praktika, o.a. de differentiatiecursus "Bepaling van geneesmiddelen en metabolieten in lichaamsvloeistoffen".
Vanaf 1972 verrichtte hij onderzoek op het gebied van de bioanalyse, farmacokinetiek en metabolisme van het geneesmiddel thiazinamium methylsulfaat. Dit onderzoek werd uitgevoerd in samenwerking met Prof. Dr. N. G. M. Orie en Drs. L. E. van Bork, Kliniek voor Inwendige Ziekten, afd. Longziekten, Academisch Ziekenhuis, Groningen. Bij dit onderzoek werd aanvankelijk voornamelijk aandacht besteed aan intraveneuze, intramusculaire en orale toediening. Later werd het uitgebreid met de bestudering van een aantal biofarmaceutische aspecten van de rectale toediening van het geneesmiddel. Dit onderzoek werd verricht in samenwerking met Drs. H. L. M. Cox, Laboratorium der Nederlandse Apothekers, Den Haag. In 1974 was hij enige tijd werkzaam aan het Laboratorium voor Farmaceutisch-Analytische Chemie van de Universiteit van Uppsala, Zweden, waar o.l.v. Prof. Dr. G. Schill diverse facetten van ionpaar-extractie werden bestudeerd. Als lid van de werkgroep "Bioanalyse en farmacokinetiek van geneesmiddelen in gebruik bij CARA" was hij betrokken bij de ontwikkeling van een bepalingsmethode voor oxyfenonium bromide in plasma (Drs. J. E. Greving). Samen met Drs. H. G. M. Westenberg, Laboratorium voor Farmaceutische en Analytische Chemie, Rijksuniversiteit, Groningen en Dr. E. van der Kleijn, St. Radboud Ziekenhuis, afd. Klinische Farmacie, Katholieke Universiteit, Nijmegen, verrichtte hij een onder-

zoek naar de farmacokinetiek van thiazinamium methylsulfaat en van carbamazepine in honden en werd de distributie van thiazinamium methylsulfaat, promethazine hydrochloride en carbamazepine in apen en muizen bestudeerd met behulp van autoradiografie.
Het grootste deel van de tot nu toe verrichte onderzoekingen is beschreven in dit proefschrift.
Hij is getrouwd en heeft een dochter.
Uit de genoemde onderzoekingen zijn de volgende publicaties voortgekomen:

J. H. G. Jonkman: Kwantitatieve analyse van thiazinamium methylsulfaat (Multergan®) in lichaamsvloeistoffen, *Pharm. Weekblad 109* (1974) 1095

J. H. G. Jonkman, J. Wijsbeek, S. Hollenbeek Brouwer-de Boer and R. A. de Zeeuw: Bioavailability of the quaternary ammonium compound thiazinamium methylsulphate (Multergan®) after oral and intramuscular administration. *J. Pharm. Pharmacol. 26* (1974) 63P

R. A. de Zeeuw, J. H. G. Jonkman and F. J. W. van Mansvelt: Plasticizers as contaminants in high-purity solvents: A potential source of interference in biological analysis. *Anal. Biochem. 67* (1975) 339

J. H. G. Jonkman: Ionpaar-extractie als isoleringsmethode bij de analyse van geneesmiddelen en metabolieten in lichaamsvloeistoffen. I. Inleiding en algemene principes. *Pharm. Weekblad 110* (1975) 649

J. H. G. Jonkman: Ionpaar-extractie als isoleringsmethode bij de analyse van geneesmiddelen en metabolieten in lichaamsvloeistoffen. II. Praktische uitvoering en toepassingen. *Pharm. Weekblad 110* (1975) 673

J. H. G. Jonkman, J. Wijsbeek, S. Hollenbeek Brouwer-de Boer, R. A. de Zeeuw, L. E. van Bork and N. G. M. Orie: Determination of low concentrations of the quaternary ammonium compound thiazinamium methylsulphate in plasma and urine. *J. Pharm. Pharmacol. 27* (1975) 849

L. E. van Bork, J. H. G. Jonkman, N. G. M. Orie and R. A. de Zeeuw: Thiazinamium methylsulphate; a drug with anticholinergic and antihistaminic properties; its use in chronic generalized obstructive lung disease. *Tubercle 56* (1975) 244

J. H. G. Jonkman, R. A. de Zeeuw, L. E. van Bork and N. G. M. Orie: Bioavailability after intramuscular injection. *Lancet i*, (1976) 693

J. H. G. Jonkman, J. Wijsbeek, J. E. Greving, R. E. M. van Gorp and R. A. de Zeeuw: Determination of thiazinamium sulphoxide in urine by means

STELLINGEN

I

Bij het uitvoeren van farmacokinetisch onderzoek is een gedegen kennis van de fundamentele aspecten van de bioanalyse onontbeerlijk.

II

Farmacokinetische parameters verkregen met behulp van computerprogramma's dienen met zorg te worden geïnterpreteerd.

III

De benaming „stikstof-detector" (= „nitrogen detector") voor een gaschromatografische detector, berustend op het principe van alkali vlam ionisatie, doet ten onrechte vermoeden dat deze detector uitsluitend gevoelig is voor stikstof bevattende verbindingen.

Maier-Bode, H. en M. Riedmann (1975), in: *Residue Reviews,* Vol. 54 (F. A. Gunther en J. Davies Gunther, Eds.), Springer Verlag, New York, Berlin.

IV

Het optreden van een plateauvormige plasmaconcentratie-tijdcurve na orale toediening van thiazinamium methylsulfaat kan bij chronische medicatie eventuele variaties in de biologische beschikbaarheid voor een belangrijk deel compenseren.

Dit proefschrift.

V

De opvatting dat geneesmiddelen na rectale toediening de algemene circulatie kunnen bereiken zonder voorafgaande leverpassage is in zijn algemeenheid niet juist.

Dit proefschrift.

VI

Bij het geven van een verklaring voor de discrepantie tussen de uitkomsten van hun eigen experimenten en die, welke verkregen waren door Hansson en Schmitterlöw, hebben Huang en medewerkers onvoldoende aandacht besteed aan de biofarmaceutische aspecten van hun experimenten.

Huang, C. L., J. A. Yeh en S. Y. Hsu (1970), *J. Pharm. Sci. 59,* 772.
Hansson, E. en C. G. Schmitterlöw (1961), *Arch. Int. Pharmacodyn. 131,* 309.

VII

Gezien de zeer geringe mate waarin thiazinamium methylsulfaat de bloed-hersenbarrière passeert, is het niet waarschijnlijk dat beïnvloeding van centraal-nerveuze functies een aanleiding vormt om dit geneesmiddel bij aflevering te voorzien van de waarschuwing „Dit geneesmiddel kan de rijvaardigheid beïnvloeden".

„Herziene lijst betreffende geneesmiddelen en verkeersveiligheid behorende bij het zwaarwegende advies van K.N.M.G. en K.N.M.P." (1973).

VIII

Teneinde verschraling van de universitair-farmaceutische vorming der Nederlandse apothekers te voorkomen, dient men bij de benoeming van docenten aan de Subfaculteiten Farmacie er voldoende zorg voor te dragen dat een goede interactie tussen de algemene farmaceutische praktijk en de opleiding tot apotheker wordt gewaarborgd.

IX

Een grotere kennis omtrent de werking van geneesmiddelen bij de gebruiker ervan kan leiden tot een meer selectieve geneesmiddelenconsumptie en een grotere „patient compliance".

X

Het in der loop der jaren gegroeide gebruik van promovendi in de Subfaculteiten Farmacie om in de vorm van een stelling, behorend bij hun proefschrift, kritiek te uiten op de Nederlandse Farmacopee zonder deze kritiek gemotiveerd onder de aandacht van de Farmacopee-commissie te brengen, moet als weinig opbouwend worden beschouwd.

XI

Door tranquillizers en hypnotica uitsluitend in de vorm van suppositoria beschikbaar te stellen aan diegenen, die aan deze medicamenten zijn verslaafd, kan overconsumptie worden vermeden.

XII

Uit het feit, dat de ziekenfondsen in het algemeen geen vergoeding geven voor een pruik voor mannen, maar wel voor vrouwen, blijkt een miskenning van de ijdelheid van de man.

XIII

Tegen de indringende reclame van de farmaceutische industrie voor hun geneeskrachtige synthetische producten is geen kruid gewassen.

XIV

Uit het feit, dat „auto" vaak één der eerste woorden is in de vocabulaire van een kind, blijkt de belangrijke plaats die dit vervoermiddel in de huidige maatschappij inneemt.

J. H. G. JONKMAN

Groningen, 20 juni 1977.

of Amberlite XAD-2®-column chromatography and thin-layer densitometry. *J. Chromatog. 128* (1976) 208

J. H. G. Jonkman, L. E. van Bork, J. Wijsbeek, R. A. de Zeeuw en N. G. M. Orie: De biologische beschikbaarheid van de kwaternaire ammonium verbinding thiazinamium methylsulfaat (Multergan®) na verschillende wijzen van toediening bij de mens. *Pharm. Weekblad 111* (1976) 1209

J. H. G. Jonkman, J. Wijsbeek, R. A. de Zeeuw, L. E. van Bork and N. G. M. Orie: Variations in the bioavailability of thiazinamium methylsulfate. *Clin. Pharmacol. Ther. 21* (1977), 457

J. H. G. Jonkman, H. G. M. Westenberg and E. van der Kleijn: A comparison of the distribution of promethazine and its quaternary analogue in mice and monkey. *Acta Pharmacol. Toxicol. 41, Suppl. 1* (1977) 124

E. van der Kleijn, N. V. M. Rijntjes, T. B. Vree, F. Schobben, H. G. M. Westenberg, J. H. G. Jonkman, H. J. Knop and H. van Geyn: Similarities and differences in the kinetics of distribution and of placental transfer of antiepileptic drugs of extremely varying physico-chemical properties. *Acta Pharmacol. Toxicol. 41, Suppl. 1* (1977) 168

H. G. M. Westenberg, J. H. G. Jonkman and E. van der Kleijn: The distribution of carbamazepine and its metabolite in squirrel monkey and mouse. *Acta Pharmacol. Toxicol. 41, Suppl. 1* (1977) 136

L. E. van Bork, J. H. G. Jonkman, R. A. de Zeeuw, N. G. M. Orie, R. Peset en K. de Vries: Diagnostisch en therapeutisch gebruik van thiazinamium methylsulfaat (Multergan®). (Is voor publicatie geaccepteerd door de redactie van het Nederlands Tijdschrift voor Geneeskunde)

Appendix 1

Appendix 1

Equations used for the calculations of pharmacokinetic parameters (N.B. All processes were assumed to occur in a first-order fashion).

$$(t_{\frac{1}{2}})_{\alpha} = \frac{0.693}{\alpha} \qquad (t_{\frac{1}{2}})_{\beta} = \frac{0.693}{\beta}$$

$$k_{12} = \frac{A \cdot B \cdot (\beta - \alpha)^2}{c_p^o \cdot (A \cdot \beta + B \cdot \alpha)} \qquad k_{21} = \frac{A \cdot \beta + B \cdot \alpha}{c_p^o}$$

$$k_{13} = \frac{c_p^o}{A/\alpha + B/\beta} \qquad (V_d)_{\beta} = \frac{D^* \cdot \alpha}{A \cdot \beta + B \cdot \alpha}$$

$$V_c = \frac{D^*}{c_p^o} \qquad Cl_{tot} = k_{13} \cdot V_c$$

where:

A = intercept of the calculated (back-extrapolated) monoexponentially declining line of the first phase (α-phase) with the ordinate (ng/ml).

α = slope of the line mentioned above (hybrid constant), also called distribution rate constant (min.$^{-1}$).

B = intercept of the calculated (back-extrapolated) monoexponentially declining line of the second phase (β-phase) with the ordinate (ng/ml).

β = slope of the line mentioned above (hybrid constant), also called overall elimination rate constant (min.$^{-1}$).

c_p^o = $A + B$ (ng/ml).

$(t_{\frac{1}{2}})_{\alpha}$ = α-phase half-life (min.).

$(t_{\frac{1}{2}})_{\beta}$ = β-phase half-life, considered as biological half-life (min.).

k_{12} = distribution rate constant for transfer of thiazinamium cations from the central to the peripheral compartment (min.$^{-1}$).

k_{21} = distribution rate constant for transfer of thiazinamium cations from the peripheral to the central compartment (min.$^{-1}$).

k_{13} = elimination rate constant of thiazinamium cations (min.$^{-1}$).

V_c = apparent volume of distribution of the central compartment. Also indicated as V_1 (1 or 1/kg).

$(V_d)_{\beta}$ = apparent volume of distribution of the peripheral compartment. Also indicated as V_2 (1 or 1/kg).

Cl_{tot} = total clearance = the clearing of a hypothetical plasma volume of a drug (ml/min.).

D^* = dose administered (theoretical amount), expressed as thiazinamium methylsulphate (mg or mg/kg).

Other symbols used in Part III are:

AUC = area under the plasma concentration-time curve (generally arbitrary units have been used).

F = bioavailability as fraction of the dose (%).

t_o = lag-time (time between the moment of administration of the drug and the beginning of the absorption process).

k_a = absorption rate constant (min.$^{-1}$).

$(t_{\frac{1}{2}})_a$ = absorption half-life (min.).

t_{max} = the time after which the maximum plasma concentration is reached (min.).

c_{max} = the maximum plasma concentration of the drug during an experiment (ng/ml).