EUROPEAN JOURNAL OF DRUG METABOLISM AND PHARMACOKINETICS, 1984, Vol. 9, No 1, pp. 17-29

The fate of Drotaverine-Acephyllinate in rat and man II. Human pharmacokinetics of Drotaverine-^{14}C-Acephyllinate

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Received for publication : March 26, 1982

Key words : Depogen, volunteer, oral route, pharmacokinetics, mathematical modelling

SUMMARY

Pharmacokinetics of Drotaverine-Acephyllinate, Chinoin was investigated in seven male volunteers using ^{14}C labelled drug. Drotaverine-Acephyllinate was administered at a 100 mg single oral dose. Measurements of total radioactivity showed that the drug was absorbed completely and was eliminated by renal and biliary routes. Within 72 hours 39.9 ± 9.9% and 47.1 ± 4.9% of the dose were recovered in the urine and faeces respectively. Experimental results were interpreted on the basis of a complex linear compartment model. The structural identifiability of the model was proved by computer analysis, and the pharmacokinetic parameters were determined.

INTRODUCTION

Drotaverine-Acephyllinate, Depogen[+] (Fig. 1) (6,7,3′,4′-tetraethoxy-1-benzyl-3,4-dihydro-isoquinoline theophylline-7-acetate) was synthesized by Szentmiklósi and Mészáros (1). The compound is a potential new drug having more favourable pharmacological properties than the other common salts of Drotaverine, Chinoin (2, 3). Preclinical and clinical investigations showed considerably decreased vascular resistance in the extremities obviously due to a powerful peripheral vasodilator and excellent spasmolytic effect (4) of the drug.

Drotaverine-Acephyllinate is the theophylline-7-acetic acid salt of Drotaverine (6,7,3′,4′-tetraethoxy-1-benzyl-3,4-dihydro-isoquinoline) and since in a water solution it dissociates to Drotaverine and theophylline-7-acetic acid, the pharmacokinetics and metabolism of these moieties can be examined separately.

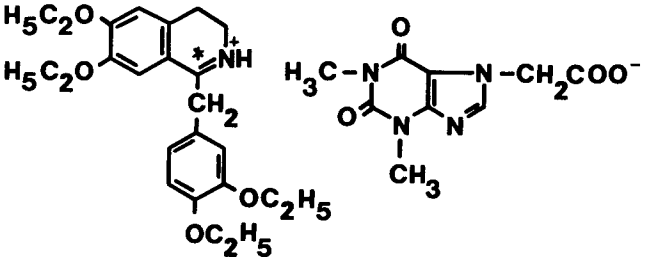

Fig. 1 : The structure of Drotaverine-Acephyllinate (*position of ^{14}C label)

Zuidema (5,6) worked out a rapid specific method for the high-performance liquid-chromatographic determination of acephylline (Theophylline-7-acetic acid) in human serum and the gas-liquid chromatographic determination of acephylline in urine. A volunteer was injected intravenously with acephylline piperazine (500 mg/5 ml) (7) and after 4 hours the total dose of acephylline had been recovered from the urine. After oral administration of 500 mg

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acephylline piperazine to volunteers no acephylline was detected in the serum and after 8 hours 1.4% of the total dose of acephylline was found in the urine. When the urine was hydrolysed by acid and heat, the maximum concentration of acephylline was 2.4% and no other xanthine derivatives were found. These experiments indicated that acephylline given intravenously was completely eliminated in the urine within 4 hours and oral absorption was very poor, as in animals (8).

The present investigation was based on the pharmacokinetic studies of papaverine (9-12) and the preliminary results of Drotaverine and Drotaverine-Acephyllinate (13-7). Rutz-Coudray (18) studied the pharmacokinetics of Drotaverine-^{14}C in man and the results were based on the total radioactivity of blood, urine and faeces regardless of the metabolic pathways. Our investigations were aimed at collecting detailed information on the pharmacokinetic and metabolic changes of Drotaverine-Acephyllinate in the human organism using the Drotaverine-Acephyllinate in Drotaverine-1-^{14}C labelled orm.

MATERIALS AND METHODS

Drotaverine-Acephyllinate (Drotaverine-1-^{14}C)

Drotaverine-1-^{14}C was synthesized by Koltai and coworkers (19) Laboratory of Radiochemistry, Institute for Drug Research, Budapest and this compound was used for the preparation of Drotaverine--Acephyllinate, Depogen-^{14}C (Fig. 1) (the specific activity of Depogen-^{14}C was 0.268 GBq/g (7.255 mCi/g), 0.170 GBq/mM (4.612 mCi/mM). The radiochemical purity of Drotaverine-Acephyllinate--^{14}C was checked by thin layer chromatography on Kieselgel G (Merck) layer (0.2 mm thick), using n-buthanol:ethanol:water (4:4:1 V/V) or benzene acetone:methanol (7:2:1 V/V) solvent system. The quantitative determinations of the radioactive spots were made on the chromatograms with a Berthold LB 2723 scanner. The radiochemical purity of Drotaverine-Acephyllinate-^{14}C proved to be $>95\%$ in both solvent systems.

The drug was administered in hard gelatine capsules, each containing 100 mg Drotaverine-Acephyllinate (total radioactivity 3.7 MBq) plus 100 mg vehicle. This corresponded in every respect to the standard Chinoin 100 mg Drotaverine-Acephyllinate capsule.

SUBJECTS

Seven male volunteers with normal renal function and with no hepatic or cardiovascular diseases participated in the study. The data regarding the subjects and the dose of the drug are shown in Table I. None of the subjects had taken any other drug for

Table I : Data of human volunteers treated with Drotaverine-^{14}C-Acephyllinate.

Name	Age	Body w.	Height	Body surface	Dose		Tot. act.
		(kg)	(cm)	(m^2)	mg	μmol	MBq
K.F.S.	40	85	180	2.05	100.2	157.6	3.7
K.GY.	40	78	170	1.9	100.5	158.1	3.7
B.S.	40	66	170	1.8	99.6	156.7	3.76
SZ.T.	37	84	187	2.0	100.0	157.3	3.7
V.Z.	39	68	170	1.8	100.6	158.2	3.7
CS.J.	47	71	175	1.9	100.0	157.3	3.7
K.M.	48	74	176	1.9	100.0	157.3	3.7

a week before and in the course of the investigations. Before administration, they underwent complete laboratory tests including blood count, serum urea, serum creatinine, total protein, albumin, α_1 globulin, α_2 globulin, β globulin, total bilirubin, SGOT, SGPT, SGGTP, alkaline phosphatase and total urine analysis.

Experimental conditions

The subjects took the drug on empty stomach in gelatine capsules with a glass of water. Two hours after administration each subject had breakfast and subsequently had normal meals. Blood pressure, pulse rate and general condition of the subject were checked at regular intervals.

Treatment of Samples

Blood samples were taken before administration (control sample), and at 15, 30, 45 minutes, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, 30, 48 and 72 hours through a teflon cannula fixed in a cubital vein into heparinized centrifuge tubes (10 IU/ml blood in isotonic NaCl). The samples were centrifuged at 3000 r.p.m. for 20 minutes, plasma and blood cells were separated and stored at -20°C until use.

Urine was collected separately at 0-2, 2-4, 4-6, 6-8, 8-10, 10-24, 24-30, 30-48, 48-72 hours after drug administration. 50-100 ml of each sample was placed into 10 ml ampoules (with 10 µl chloroform added per ampoule), sealed and kept at -20°C until use.

The faecal samples were weighed and homogenized in a grinding mortar. 4-6 g of each sample was spread onto the bottom of a weighed Petri dish and dried to constant weight in a vacuum desiccator over P_2O_5. These samples were used for radioactivity measurements and metabolic studies.

Measurement of radioactivity

Sample preparation for liquid scintillation counting: 10 ml Aquasol® (New England Nuclear) scintillation cocktail was added to 0.2 ml urine, plasma or extraction residue. 0.1 ml whole blood or 0.05 ml of cells was mixed in the counting vial with 1.5 ml of Soluene-350® (Packard)/isopropanol, 35% hydrogen peroxide mixture (1.5 : 1.5 : 1 V/V) with gentle agitation and let stand at room temperature for 10 minutes, and for 2 hours at 40°C. After cooling 10 ml Aquasol-HCl (Aquasol : 0.5 N HCl = = 9 : 1 V/V) was added per vial.

The dry faeces samples (10-30 mg) were combusted in a stream of oxygen using an Oxiscint apparatus (20). Carbon dioxide was trapped in an absorber containing 2.0 ml Carbo-Sorb II® (Packard). This solution was rinsed into a vial 13 ml cocktail (4.0 g PPO, 0.25 g POPOP in 1 litre toluene).

The measurements were carried out with an LKB Wallac 8100 liquid scintillation spectrometer using external standard quench correction.

Determination of unchanged Drotaverine

In the biological samples the unchanged Drotaverine was determined by extraction technique as previously described (16). n-Heptane proved to be the most suitable solvent as shown by the following efficiency data obtained at pH 14.0 :

92 ± 2.2 for unchanged Drotaverine
3 - 5% for «free metabolites» (de-ethylated, i.e. free phenolic hydroxyl group containing Drotaverine derivatives)
0% for «conjugated metabolites» (glucuronide and sulphate conjugates).

Plasma, urine and supernatants (3000 r.p.m., 20 min) of aqueous faeces homogenates were extracted three times with three volumes of n-heptane at pH 14.0. The radioactivity of the extraction residues was measured by liquid scintillation counter.

Enzymic hydrolysis of conjugates

Nine ml 96% ethanol was added to 1 ml of each plasma sample, the mixture was stirred for 5 min and centrifuged at 3000 r.p.m. for 15-20 min. The clear supernatant was drawn off, 9 ml 96% ethanol was added to the sediment, and the extraction procedure was repeated. The ethanol phases were pooled and evaporated in vacuo at 40°C. The dry residue was dissolved in 2.5 ml pH 5.0 acetate buffer, 0.1 ml of the solution was pipetted into a liquid scintillation vial, 10 ml Bray cocktail was added and radioactivity was measured. The solution was separated into two parts, further on marked as Solution A and B.

Solution A

The samples were extracted with 3 × 3 volumes chloroform at pH 5.0 to give amount unchanged Drotaverine and «free metabolites». «Conjugated

metabolites» were not extractable at this pH. After separation of the phases, radioactivity of the aqueous residue was measured.

Solution B

20 µl, 2000 FU β-glucuronidase/arylsulphatase (SERVA) was added to each sample, the mixture was incubated for 24 hours at 37°C. Radioactivity of the aqueous phase was measured before and after extraction with 3 × 3 volumes of chloroform.

1 ml pH 5.0 acetate buffer was added to 1 ml urine or supernatant of the aqueous homogenate of faeces samples and the mixture was incubated in the presence of 2000 FU β-glucuronidase/arylsulphatase for 24 hours at 37°C. Extraction percentage with 3 × 3 volumes of chloroform gives the amount of conjugated metabolites. To differentiate between glucuronide and sulphate conjugates, separate samples were incubated in the presence of 2000 FU β-glucuronidase (Ketodase, Gödecke AG) and treated as above.

Protein binding

Protein binding level was determined by in vitro and in vivo methods at 24°C and 37°C. In the in vitro experiments, the labelled product was incubated with human serum albumin in pH 7.4 phosphate buffer for 24 hours. For the in vivo experiments the plasma samples of the subjects were used.

In the in vivo as well as in the in vitro studies, protein binding was determined by equilibrium dialysis technique using Visking Tube® (Union Carbide) membrane.

RESULTS

Plasma levels of unchanged Drotaverine, «free metabolites» and «conjugated metabolites» were measured, using enzymic hydrolysis and extraction technique (Table II).

Fig. 2 shows the ^{14}C radioactivity levels in the plasma, whole blood and blood cells after administration of Drotaverine-Acephyllinate-^{14}C.

Fig. 3 shows the urinary and faecal elimination of radioactivity.

Of the metabolites in conjugated form excreted in the urine almost all were in the form of glucuronides while only 1-3% were sulphate conjugates.

The equilibrium dialysis showed that protein binding of Drotaverine and its metabolites to plasma proteins was between 80 and 95 percent.

Table II : Plasma concentrations of unchanged Drotaverine, free and conjugated metabolites following oral administration of Drotaverine Acephyllinate (n = 7).

Time (h)	Unchanged Drotaverine nmol/ml		«Free metabolites» nmol/ml		«Conjugated metabolites» nmol/ml	
	$\bar{x}$	s ±	x	s ±	$\bar{x}$	s ±
0.5	0.788	0.301	1.040	0.212	2.212	1.0
0.75	1.088	0.236	1.041	0.241	3.305	0.646
1	1.033	0.334	0.770	0.258	3.522	0.487
1.5	0.817	0.288	0.775	0.381	2.961	0.418
2	0.612	0.318	0.576	0.220	2.815	0.412
3	0.454	0.326	0.350	0.126	2.334	0.237
4	0.301	0.253	0.361	0.143	2.026	0.190
5	0.228	0.208	0.204	0.092	1.992	0.310
6	0.175	0.161	0.149	0.121	2.016	0.223
8	0.079	0.084	0.149	0.127	1.615	0.319

Mathematical modelling

The model was established by using the following experimental results and conclusion :

- The fact that de-ethylated and conjugated metabolites appeared in the plasma simultaneously with unchanged compound, indicated a considerable hepatic «first pass effect»
- From 6 h on following administration, practically the total plasma radioactivity was represented by conjugated metabolites
- Drotaverine appeared in faeces and urine exclusively in form of conjugated metabolites
- Between 6 and 8 h the plasma concentration curve of the conjugated metabolites showed a break in the slope, indicating a slower elimination rate, and in a few cases even a low second peak occured
- The renal clearance of the conjugated metabolites calculated from the plasma and urine curves, tended to decrease with time, and by the end of the experiments declined to nearly one third of the initial value
- About 90% of the administered radioactivity appeared in the urine and faeces within 72 h
- Comparison of plasma and whole blood ^{14}C levels indicated that only a negligible fraction of the labelled compounds was bound to blood cells.

The kinetic model was formulated by a first order differential equation system representing a compartmental scheme of absorption, distribution, biotransformation and elimination events. The schematic view of the model is shown in Fig. 4.

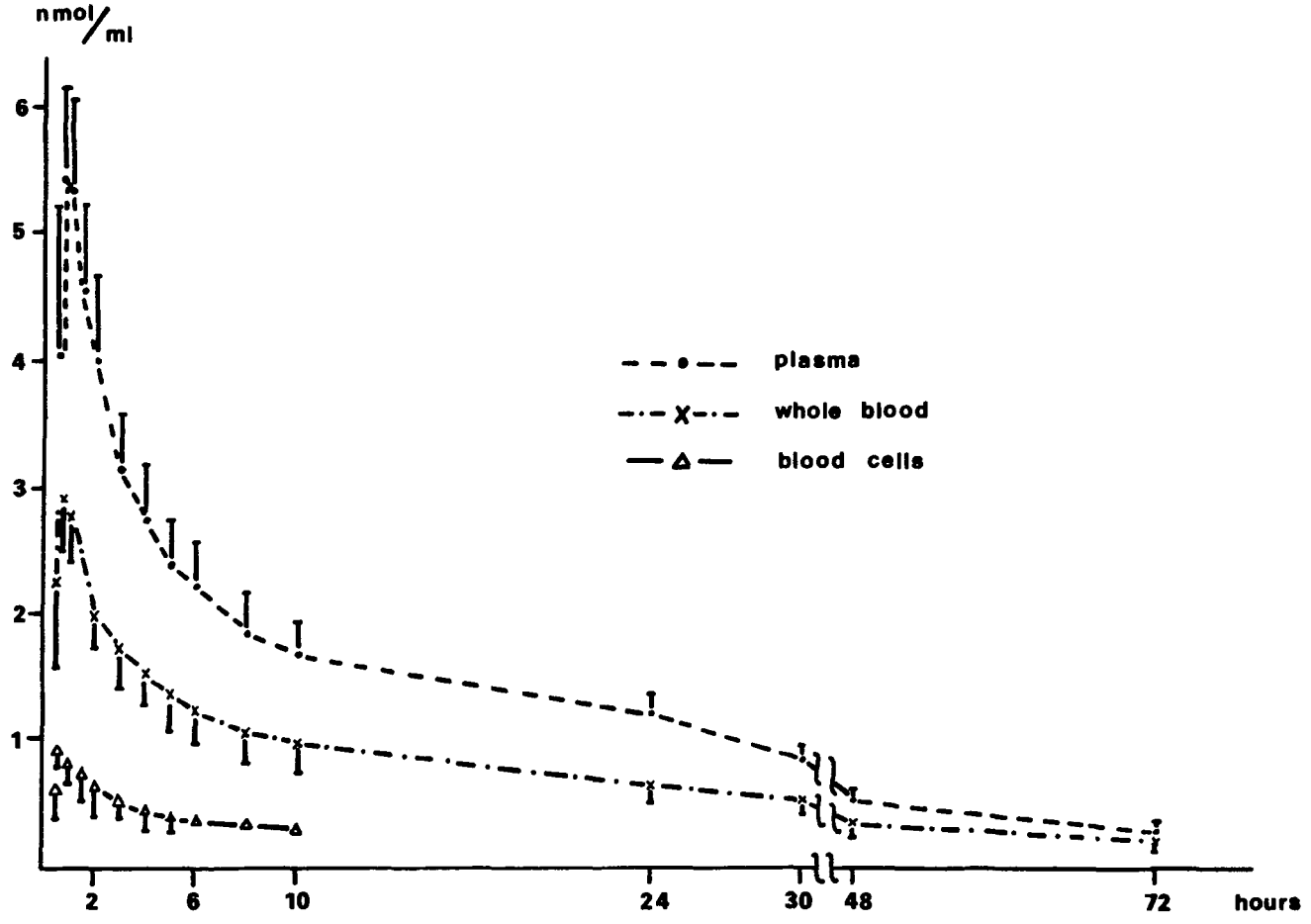

Fig. 2 : Plasma, whole blood and blood cells ^{14}C levels after administration of Drotaverine-^{14}C-Acephyllinate (the radioactivity results expressed in nmol/ml, n = 7).

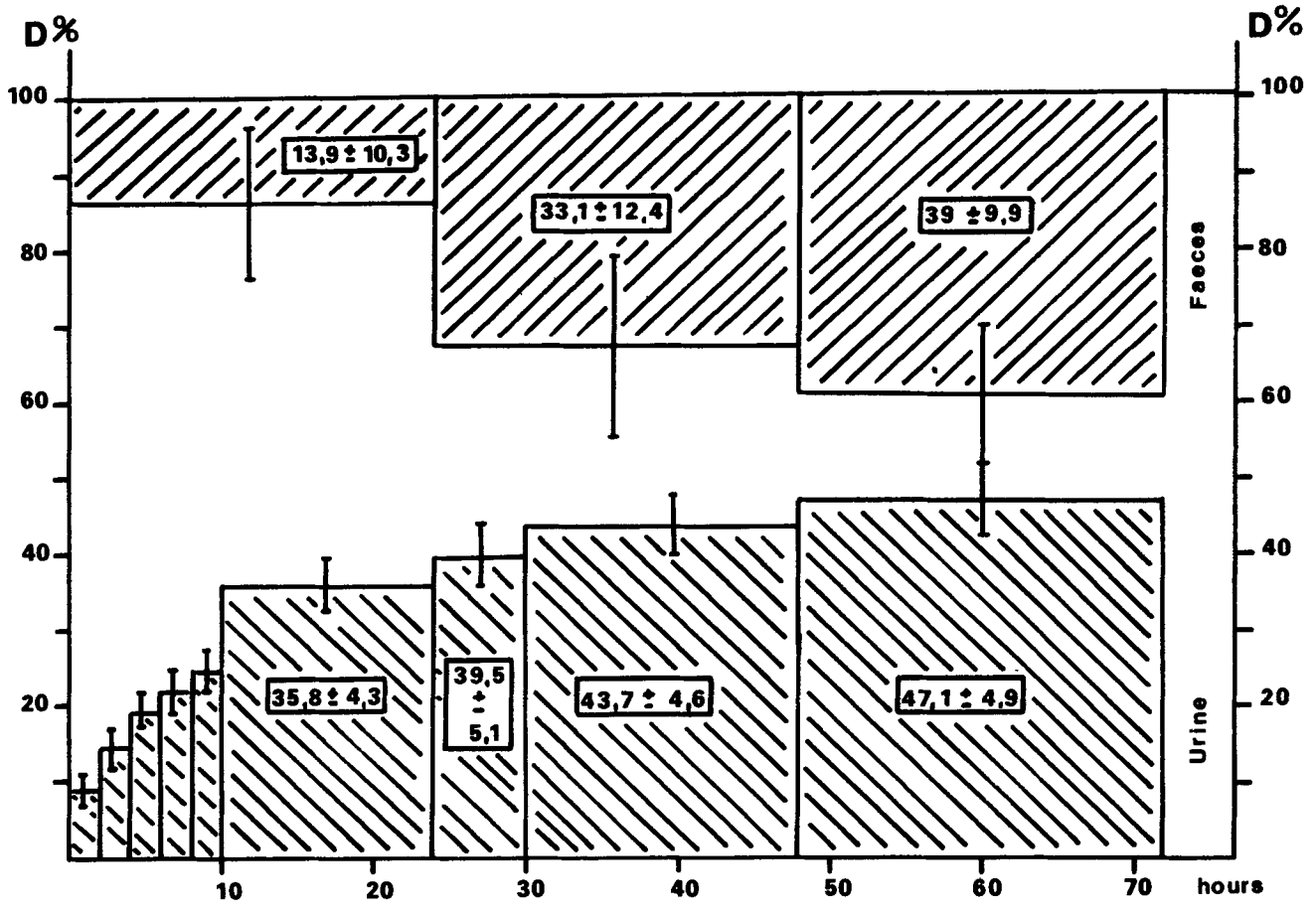

Fig. 3 : Cumulative urinary and faecal elimination of ^{14}C in % of the dose (n = 7).

Symbols :

m_A : amount of the drug at the absorption site

T_{lag} : lag time of absorption

$m^1_D, m^1_M, m^1_{K2}, m^1_{K1}$: amount of unchanged Drotaverine de-ethylated metabolites, mono- and di-conjugated metabolites in the central compartment

$m^2_D, m^2_M, m^2_{K1}, m^2_{K2}$: amount of unchanged Drotaverine, de-ethylated metabolites, mono- and di-conzugated metabolites in the peripheral compartment

m_U : cumulative amount of conjugated metabolites

m_{ENT1}, m_{ENT2} : amount of conjugated metabolites in the hypothetic enterohepatic circulation

V_D, V_K : central distribution volume of the Drotaverine and conjugated metabolites

P .... first order rate constants

F_1 : fraction of the total absorbed quantity which entered the systemic circulation in the form of unchanged Drotaverine in the course of the first liver pass

F_2 : fraction of the de-ethylated metabolites which was transformed to conjugates during the first liver pass

ENT : fraction of conjugated metabolites entering the enterohepatic circulation

TE : time constant of enterohepatic circulation

$\div$: divisor

$\sum$: Summator

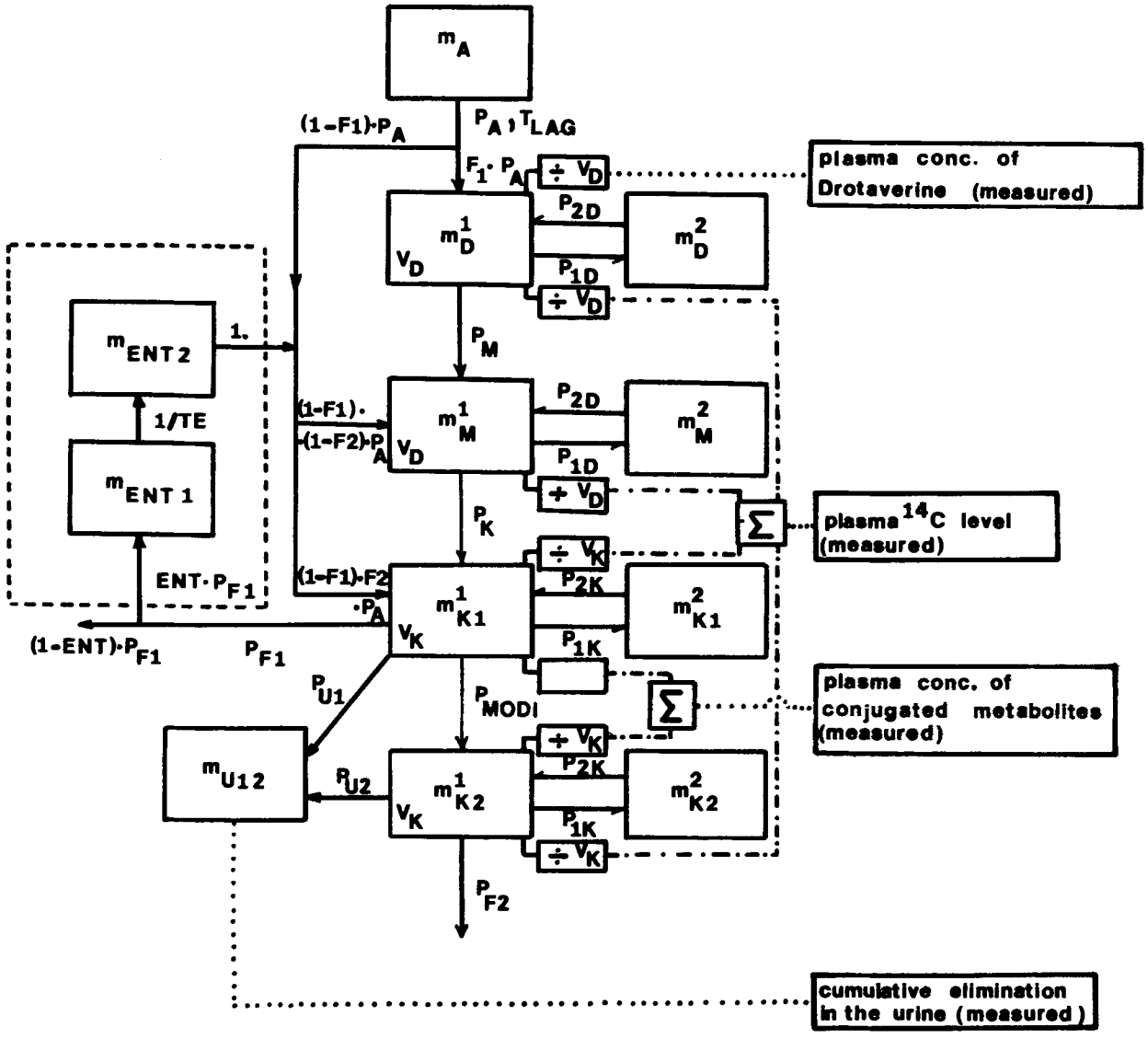

Fig. 4 : The complex linear model of the route and structural changes of Drotaverine-Acephyllinate in the human organism.

As it is shown in Fig. 4, the number of chemically different components were grouped in four kinetically distinguishable compartments :

unchanged Drotaverine (D)
de-ethylated metabolites (M)
mono-conjugates (K1)
di-conjugates (K2)

Differentiation between mono- and di-conjugates was necessitated by the renal clearance which can be explained by the greater molecular size of the di--conjugates. Some simplifying assumptions were used in the model formulation supposing identical distribution volumes and kinetics for D and M for K1 and K2 respectively, so the disposition kinetics was characterized by the V_D, V_K, P_{1D}, P_{2D}, P_{1K} and P_{2K} constants.

On the basis of the animal experiments, the existence of an enterohepatic cycle seemed to be probable (15). For modelling this speculative process two serially connected events of the enterohepatic cycle were postulated. The cycle was characterized by TE time constant and ENT which measured the fraction of the mono- and di-conjugates participating in the de-ethylation and reabsorption process.

The structural identifiability of the complex linear model was estimated and proved by the algorism of Vajda (21) prior to fitting.

The parameter estimation of the elaborated model was performed using the NONLIN program (22).

Two alternative models were identified for each subject. Because of the great uncertainty concerning the enterohepatic cycle, Model 1 did not take into account this process and used two different faecal elimination rate constants (P_{F1}, P_{F1}) to describe the kinetic events. The estimated parameters for the subjects are shown in Table III. The enterohepatic

Table III : Model parameters following administration of Drotaverine-^{14}C-Acephyllinate (n = 7).

Parameter	Subject							
	K.F.S.	K.Gy.	B.S.	Sz.T.	V.Z.	Cs.J.	K.M.	mean (n = 7)
P_A h^{-1}	2.53	3.24	2.06	2.97	3.08	2.73	1.76	2.54
	(1.92)	(2.95)	(1.87)	(1.86)	(2.45)	(2.29)	(2.04)	(0.80)
T_{LAG} h^{-1}	0.23	0.20	0.40	0.15	0.13	0.15	0.20	0.172
	(0.20)	(0.17)	(0.28)	(0.20)	(0.11)	(0.10)	(0.19)	($5.7.10^{-2}$)
F1	0.31	0.37	0.41	0.42	0.53	0.50	0.45	0.40
	(0.22)	(0.27)	(0.32)	(0.22)	(0.42)	(0.37)	(0.26)	(0.104)
F2	0.65	0.64	0.80	0.68	0.81	0.79	0.78	0.69
	(0.09)	(0.08)	(0.11)	(0.18)	(0.21)	(0.34)	(0.23)	($4.7.10^{-2}$)
P_M h^{-1}	0.84	0.31	0.55	0.62	0.79	1.35	1.07	0.62
	(0.47)	(0.25)	(0.37)	(0.42)	(0.29)	(0.68)	(0.77)	(0.142)
P_{1D} h^{-1}	0.43	1.00	0.73	1.10	0.96	1.06	0.41	0.80
	(0.35)	(0.76)	(0.43)	(0.27)	(0.43)	(0.59)	(0.37)	(0.15)
P_{2D} h^{-1}	0.53	2.61	1.11	2.63	0.69	0.71	0.58	0.72
	(0.44)	(1.17)	(0.87)	(2.11)	(0.49)	(0.28)	(0.37)	(0.178)
P_K h^{-1}	0.86	0.87	0.64	1.12	0.98	0.59	0.69	0.77
	(0.21)	(0.25)	(0.21)	(0.49)	(0.84)	(0.29)	(0.34)	(0.107)
P_{1K} h^{-1}	0.60	0.60	0.58	0.46	0.49	0.61	0.58	0.58
	(0.12)	(0.19)	(0.23)	(0.30)	(0.53)	(0.32)	(0.27)	($8.05.10^{-2}$)
P_{2K} h^{-1}	0.19	0.21	0.15	0.28	0.22	0.13	0.12	0.18
	(0.05)	(0.03)	(0.06)	(0.16)	(0.14)	(0.05)	(0.09)	($2.04.10^{-2}$)
P_{MODI} h^{-1}	0.41	0.45	0.51	0.37	0.52	0.43	0.57	0.44
	(0.37)	(0.40)	(0.55)	(0.28)	(0.37)	(0.33)	(0.44)	(0.140)
P_{U1} h^{-1}	0.21	0.20	0.24	0.16	0.27	0.21	0.32	0.2
	(0.03)	(0.03)	(0.04)	(0.03)	(0.09)	(0.05)	(0.09)	($1.47.10^{-2}$)
P_{U2} h^{-1}	0.061	0.057	0.076	0.051	0.087	0.069	0.102	0.063
	(0.007)	(0.007)	(0.011)	(0.017)	(0.034)	(0.015)	(0.037)	($4.13.10^{-3}$)
P_{F1} h^{-1}	0.19	0.18	0.15	0.20	0.19	0.17	0.19	0.18
	(0.13)	(0.19)	(0.28)	(0.24)	(0.45)	(0.28)	(0.49)	($8.48.10^{-2}$)
P_{F2} h^{-1}	0.046	0.053	0.045	0.046	0.064	0.052	0.096	0.05
	(0.054)	(0.076)	(0.142)	(0.070)	(0.142)	(0.137)	(0.256)	($3.36.10^{-2}$)
V_D lit	29.5	26.3	23.8	35.8	36.1	35.9	25.4	29.35
	(7.6)	(4.8)	(5.5)	(8.9)	(7.6)	(5.7)	(6.8)	(2.38)
V_K lit	14.5	11.2	11.3	19.8	11.3	10.0	10.8	11.83
	(2.3)	(1.3)	(1.6)	(3.8)	(4.1)	(2.4)	(2.9)	(0.793)

cycle was characterized by ENT and TE parameters in Model 2. The comparison of Model 1 and Model 2 is demonstrated in Table IV for subject 1.

It should be noted that there was a great individual variability in the model parameters relating the kinetics of the mono- and di-conjugates, however this great variability can be easily interpreted by the fact that no selective measurements were available for the mono- or di-conjugates, only for their sum during the investigations. Although to describe the time decrease of renal clearance, differentiation was necessary between mono- and di-conjugates, their biotransformation, enterohepatic cycle and elimination could not been described mathematically by a unique and statistically well-defined parameter vector.

Fig. 5, 6, 7, 8 show the measured and simulated curves for subject 1. For every subject, a statistically identical fit was produced using the above mentioned two-model variants.

Table IV : Comparison of model variants 1 and 2 by parameters determined in N° 1 volunteer (The parameters pertaining to the kinetics of unchanged Drotaverine were identical in both model variants and correspond to those shown in Table III.

Parameter	Model 1	Model 2
F2	0.65 (0.09)	0.66 (0.13)
$P_K\ h^{-1}$	0.86 (0.21)	0.79 (0.19)
$P_{1K}\ h^{-1}$	0.60 (0.12)	0.59 (0.17)
$P_{2K}\ h^{-1}$	0.19 (0.50)	0.15 (0.07)
$P_{U1}\ h^{-1}$	0.21 (0.02)	0.20 (0.01)
$P_{U2}\ h^{-1}$	0.061 (0.007)	0.058 (0.003)
$P_{F1}\ h^{-1}$	0.19 (0.13)	0.096 (0.087)
$P_{F2}\ h^{-1}$	0.046 (0.054)	0.096** (0.087)
ENT	-	0.32 (1.9)
T_E h	-	2.1 (3.5)
V_K lit	14.5 (2.3)	14.8 (0.74)

** In model 2, P_{F1} and P_{F2} by definition are identical

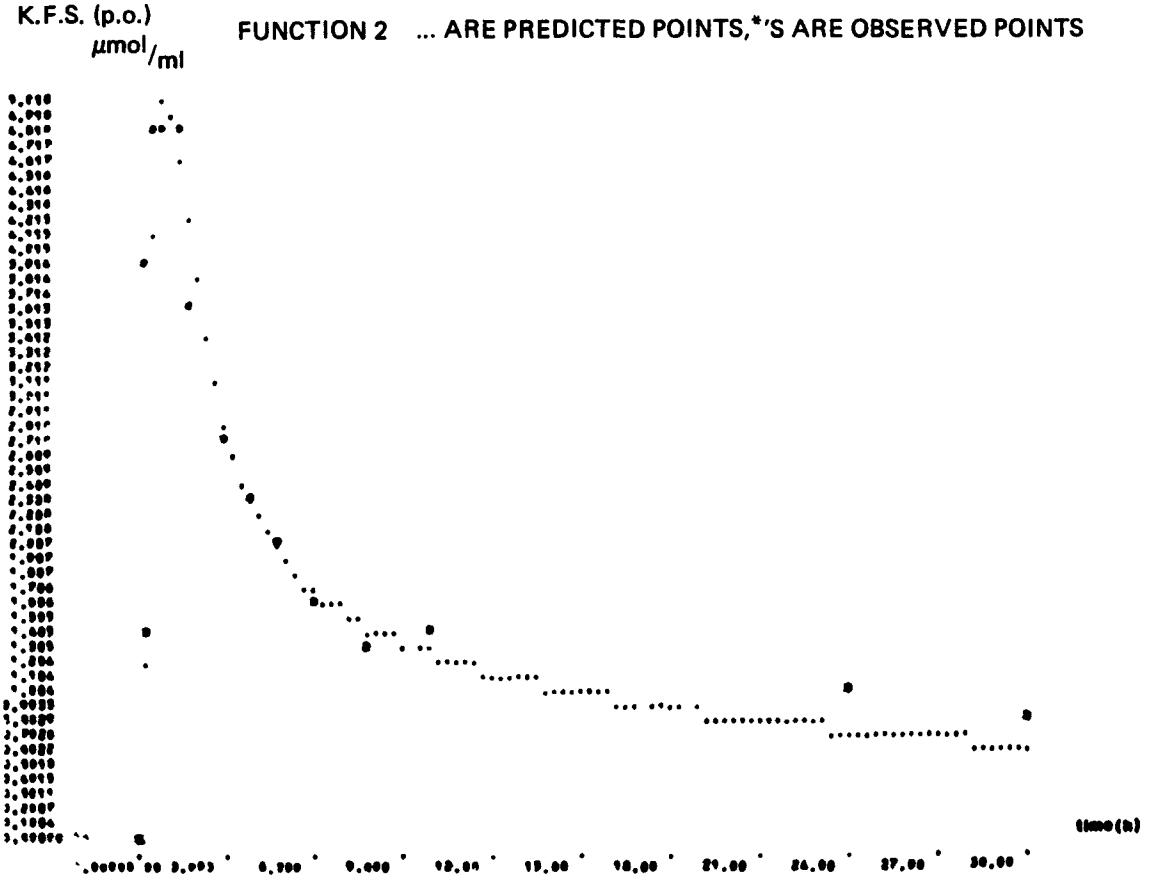

Fig. 5 : Plasma ^{14}C level after administration of 160 nmol Drotaverine-Acephyllinate (N° 1 volunteer, ... model, * experimental).

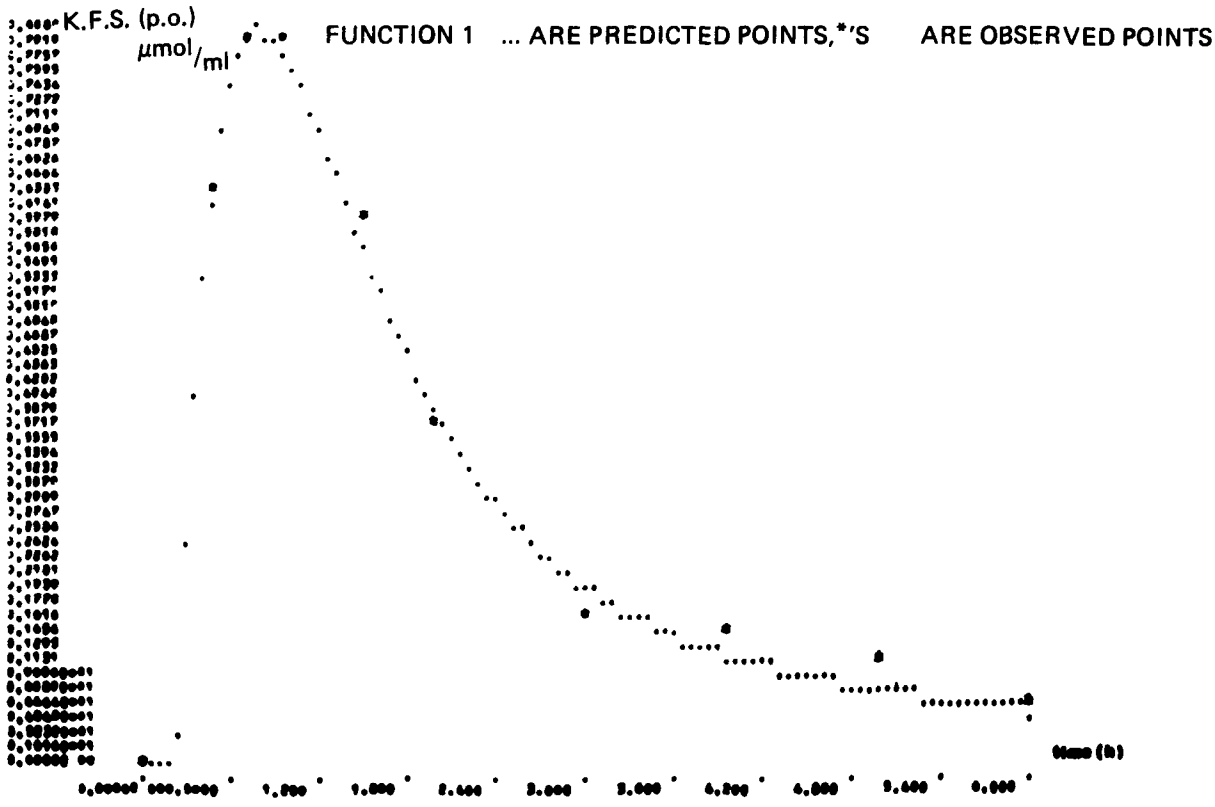

Fig. 6 : Plasma concentration of unchanged Drotaverine following administration of Drotaverine-Acephyllinate (N° 1 volunteer, ... model, * experimental).

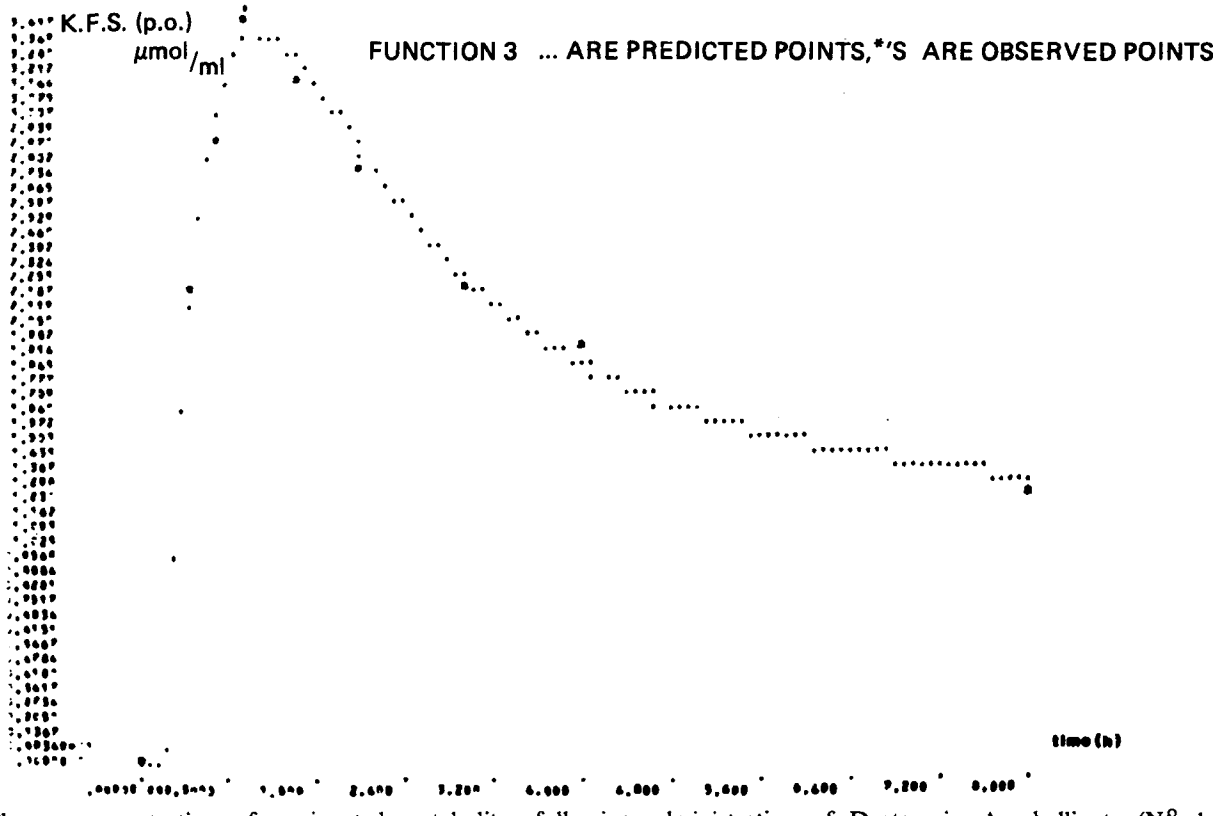

Fig. 7 : Plasma concentration of conjugated metabolites following administration of Drotaverine-Acephyllinate (N° 1 volunteer, ... model, * experimental).

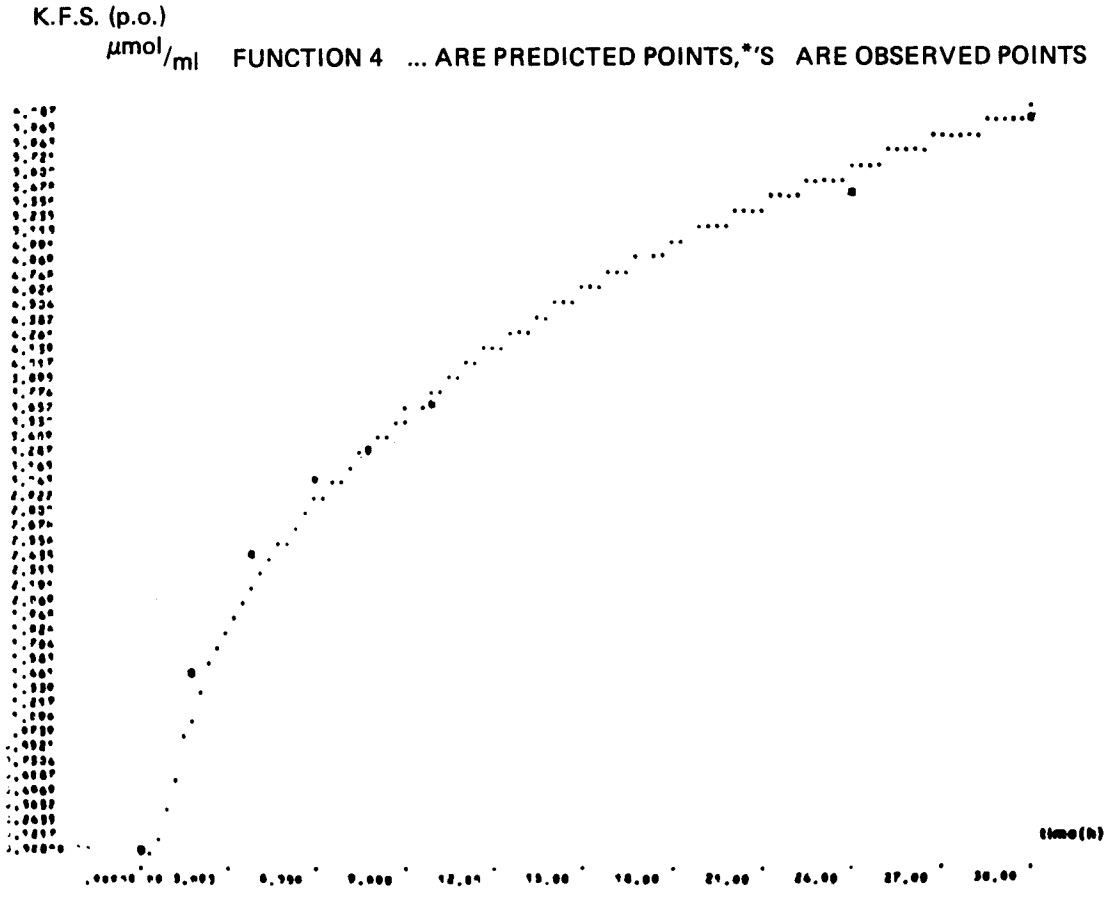

Fig. 8 : Observed and predicted data for urinary elimination of ^{14}C in % of the dose (N° 1 volunteer, ... model, * experimental).

DISCUSSION

As shown in Fig. 2 the plasma ^{14}C levels reached peak between 0.75 and 1 hours after drug administration. Comparison of levels in whole blood and plasma at identical sampling times revealed that practically all radioactivity was bound to the plasma

$$\frac{C_{plasma}}{C_{whole\ blood}} = 1.8-2.$$

Thus the blood cell binding proved to be negligible.

Drotaverine was almost completely metabolized by 0-de-ethylation to mono- and di-phenolic compounds and these metabolites conjugated rapidly with glucuronic acid in the liver.

An extraction procedure was designed for the separate determination of unchanged Drotaverine, free metabolites and conjugated metabolites in plasma. The maximum plasma levels determined by radioactivity consisted of 20 ± 5% unchanged Drotaverine, 18 ± 4 «free metabolites» and 62 ± 5% «conjugated metabolites». 8 hours after administration these values were 3-4%, 8-9% and 87-89% respectively (Table II). The relatively low concentrations of unchanged Drotaverine and «free metabolites», measured at early sampling times between (0.5 and 2 hours), have indicated a considerable first pass effect.

Fig. 2 shows ^{14}C radioactivity curves of plasma, whole blood, blood cells and plasma levels of unchanged Drotaverine, «free metabolites» and «conjugated metabolites» are shown in Table II. As can be seen, the metabolic degradation of Drotaverine and conjugation of metabolites were rapidly progressing and 8 hours after administration a very small part of the plasma radioactivity was represented by unchanged Drotaverine and «free metabolites». The ratio of conjugated metabolites tended

to increase at early sampling times and from 6-8 hours on following administration practically only conjugated metabolites were presented in the plasma. Since practically only conjugated metabolites were excreted in the urine and faeces, the degree of elimination obviously depends on the excretion of these compounds.

On the bases of mathematical modelling the following conclusions may be drawn :

- The fraction of the orally given dose (100 mg, 160 μM) of Drotaverine entering into systemic circulation after the first passage through the liver in the form of parent drug, de-ethylated metabolites and conjugates was 40%, 18% and 42%, respectively
- absorption half-life of parent compound was ~ 0.27 hours; its elimination half-life characterizing the beta phase was ~ 2.3 hours
- conjugation process of de-ethylated metabolites had a half-life of ~ 0.9 hours ($\ln 2/P_K$)
- the central and peripherial distribution volumes of parent compound and de-ethylated metabolites were nearly identical, ~ 30 litres
- central distribution volume of conjugates is less than that of mono-conjugates, ~ 12 litres
- corresponding to the differences in the molecular sizes and consequently the diffusion rates, the intercompartmental transfer constants of the conjugates were lower than the values of the non-conjugates
- ratio of renal clearences of di- and mono-conjugates was approximately 1:3
- half-life of di-conjugates in the beta phase was ~ 20 hours.

Summarizing the results of mathematical modelling it must be emphasized that question of an enterohepatic cycles existence remained unanswered. The details and mechanism of the fecal elimination (biliary recirculation and/or - after β-glucuronides hydrolysis of the conjugates - through the gut wall) could not been proved unambigously by modelling means, only two statistically equivalent solutions were given to describe these process. The elaboration of more physiological models necessitate however further experimental work especially concerning the hydrolysis of the conjugates in gastrointestinal tract and the reabsorption into systemic circulation. The further details of non-continous bile excretion seem to be very important as the reproducible break in the plasma curves at 8 hours after the drug intake coincides with the next meal periods of the investigated subjects.

ACKNOWLEDGEMENTS

The authors wish to thank Miss A. Bolehovszky, Mrs. J. Kudász, Mrs. E. Marik, Mrs. B. Nagy for their helpful technical assistance.

REFERENCES

1. Szentmiklósi P., Mészáros Z. (1973): Hung. Pat. 167.246.
2. Mészáros Z., Szentmiklósi P., Czibula I. (1962): Hung. Pat. 150.535.
3. Mészáros, Z., Szentmiklósi P., Czibula I. (1965): USA Pat. 3.337.539.
4. Szentmiklósi P., Marton S. (1979): Practical significance of drug-equivalence in preclinical research. Acta Pharmaceutical Hungarica *49*, 50-53.
5. Zuidema J., Merkus F. (1978): Rapid method for the high-performance liquid-chromatographic determination of acephylline in human serum. J. Chromatography *145*, 489-491.
6. Zuidema J., Hilbers H. (1980): Gas-liquid chromatographic determination of acephylline in urine. J. Chromatography 182 *(3-4)*, 445-7.
7. Zuidema J., Mercus F. (1980): Is acephylline a theophylline bronchodilator. Lancet, I. 1318-19.
8. Strolin-Benedetti M., Larne D., Oliver Ch., Bouvet P. (1978): Absorption and excretion of piperazine theophylline ethanoate-^{14}C in the rat. Arzneimittelforsch. *21*, 662-664.
9. Belpaire F., Bogaert M., Rosseel M. (1975): Identification of metabolites in rat bile. Xenobiotica, *5*, No. *7.*, 413-420.
10. Belpaire F., Bogaert M. (1975): Metabolism of papaverine. Species differences. Xenobiotica, *5*. No. *7.*, 421-429.
11. Belpaire F., Bogaert M. (1975): Effect of phenobarbital 3-methylcholanthrene and SKF 525-A pre-treatment in vivo and in vitro. Xenobiotica, *5*, No. *7.*, 431-438.
12. Belpaire F., Rosseel M., Bogaert M. (1978): Metabolism of paraverine. IV. Urinary elimination of papaverine metabolites in man. Xenobiotica, Vol. *8.*, *5*, 297-300.
13. Gyarmati L., Dávid A., Mészáros Z., Rácz I., Szentmiklósi P. (1975): Data on the metabolism of benzylisoquinoline derivatives. Pharmazie, *30 (3)*, 1-4.
14. Magyar K., Lengyel M., Knoll J. (1978): Absorption, distribution and elimination of Drotaverine. Acta Physiologica Acad. Scie. Hung., *51/41*, 401-411.

15. Simon G., Vargay Z., Winter M., Szüts T. (1979): The intestinal absorption and excretion of ^{14}C-drotaverine in rats. J. Drug Metab. Pharmacokinetics, *4*, 213-217.

16. Vargay Z., Simon G., Winter M., Szüts T. (1980): Qualitative and quantitative determination of Drotaverine metabolites in rat bile. Eur. J. Drug Metab. Pharmacokinetics, *5,2* 69-74.

17. Szatmári I., Vargay Z., Simon G., Szüts T. (1981): Pharmacokinetics and metabolism of Depogen in rats. Drugs, Biochemistry and Metabolism of Action, pharmacokinetics and metabolism of drugs, Mátrafüred, Hungary, 5-8 May, 1981.

18. Rutz-Coudray M., Balant L., Revillard G., Buri P. (1976): Pharmacokinetique de la drotaverine chez l'homme. Pharm. Acta Helv., *51 (9)*, 258.

19. Koltai E., Bánfi D., Engler J., Volford J., Mészáros Z. (1979): Syntheses of ^{14}C labelled drotaverinum hydrochloride. J. Labelled Compounds, XVI, *2*, 351-354.

20. Gács I., Vargay Z., Dobis O. (1980): Hung. Pat. 177.476.

21. Vajda S. (1981): Structural equivalence of linear models and compartmental systems. Mathematical Biosciences, *55*, 39.

22. Metzler C., Elfring G., McEwen A. (1976): A users manual for NONLIN and associated programs, rev. ed. The Upjohn Company, Kolamazoo, Mich.