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PHARMACOKINETIC ASPECTS OF TERT-BUTYLAMINOETHYL DISULFIDE, AN EXPERIMENTAL DRUG AGAINST SCHISTOSOMIASIS IN MICE

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A preliminary study of the pharmacokinetic parameters of t-Butylaminoethyl disulfide was performed after administration of two different single doses (35 and 300 mg/kg) of either the cold or labelled drug.

Plasma or blood samples were treated with dithiothreitol, perchloric acid, and, after filtration, submitted to further purification with anionic resin. In the final step, the drug was retained on a cationic resin column, eluted with NaCl 1M and detected according to the method of Ellman (1958). Alternatively, radioactive drug was detected by liquid scintillation counting.

The results corresponding to the smaller dose of total drug suggested a pharmacokinetic behavior related to a one open compartment model with the following parameters: area under the intravenous curve ($AUC_{i.v.}$): 671 ± 14; AUC_{oral}: 150 ± 40 μg.min.ml^{-1}; elimination rate constant: 0.071 min^{-1}; biological half life: 9.8 min; distribution volume: 0.74 ml/g.

For the higher dose, the results seemed to obey a more complex undetermined model. Combining the results, the occurrence of a dose-dependent pharmacokinetic behavior is suggested, the drug being rapidly absorbed and rapidly eliminated; the elimination process being related mainly to metabolization.

The drug seems to be more toxic when administered I.V. because by this route it escapes first pass metabolism, while being quickly distributed to tissues. The maximum tolerated blood level seems to be around 16 μg/ml.

Key words: aminodisulfides pharmacokinetics – schistosomiasis

A series of chemically similar compounds has been tested against schistosomiasis mansoni by Nelson & Pellegrino (1976). Among these compounds, tert-butylaminoethyl disulfide (TBAESS) was one of the most active and least toxic when administered orally. On the other hand, preliminary results obtained by these authors indicate that this drug is much more toxic when administered intravenously (I.V.) than when it is administered orally. Several factors would help to explain such a different behavior in the two situations:

a) A high rate of hepatic metabolism when administered orally;
b) A decreased intestinal absorption;
c) An increased rate of drug distribution when administered I.V.

The determination of pharmacokinetic parameters would help in deciding which of these factors are important in understanding the above mentioned behavior. Therefore, this paper proposes to calculate the main pharmacokinetic parameters of TBAESS in normal mice in an attempt to study the gastrointestinal (G.I.) absorption, the bioavailability and the rate of blood drug disappearance, as well as to obtain an indication of the drug blood levels necessary to induce toxicity in the mouse organism.

MATERIAL AND METHODS

Animals: male white mice weighing 20-30 g were used in these experiments. They received

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the drug either orally (300 or 35 mg/kg) or intravenously (35 mg/kg).

Drugs and reagents: TBAESS was prepared in these laboratories from t-butylaminoethanol (Aldrich) by classical methods via the intermediate aminobromide (Blatt, 1943) and isothiourunium salt (Vogel, 1951), followed by oxidation to the disulfide (Elderfield, 1947) (m.p. of hydrochloride salt = 242-4 °C).

Dithiothreitol (DTT) and 5.5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chem. Co. Perchloric acid ($HClO_4$), ethanol, hydrogen peroxide, hydrochloric acid, ethyl ether, EDTA, tris-hydroxymethylaminoethane, sodium hydroxide, sodium borate, naphthalene, anionic resin type III, 2,5-diphenyloxazole (PPO) and methanol were obtained from Merck. Cationic resin type AG 50W X_4 was purchased from Bio-Rad. Ammonium hydroxide and potassium carbonate were obtained from J. T. Baker; sodium borohydride from Carlo Erba.

Methodology

a) *Isolation and detection of the unaltered drug:*

1 – *From blood samples* – total drug (free plus bound) – After decapitation of the animals at regular intervals, blood samples were immediately taken, treated with DTT 0.6 M, submitted to protein precipitation with $HClO_4$ at a final concentration of 0.4 M and centrifuged at 21,000 g for 10 min. The supernatant was filtered, neutralized with 4 M potassium carbonate and kept overnight at –20 °C, after addition of ethanol.

Further purification of the samples was realized by shaking with a batch of anionic resin during 1h and the final step of the isolation process involved retention of the samples on cationic resin columns. The drug retained on these columns was eluted with 1 M sodium chloride solution in 50% ethanol containing EDTA (0.5 mg/ml) and detected according to the method of Ellman (1958). For detection of the free drug, the collected blood was immediately centrifuged, the resultant plasma transfered to tubes containing $HClO_4$ and the samples purified as described above for the total drug.

2 – *From gastro-intestinal contents* – Just after decapitation of the animals, a portion of G.I. tract (including stomach and small intestine) was excised, minced and incubated in $HClO_4$ (0.4 M final concentration) during 20 min at 0 °C. The samples were then centrifuged (21,000 g, – 20 °C, 10 min) and filtered in order to obtain a protein-free extract. The samples were then treated using the same procedure as described above for the blood samples.

3 – *From urine samples* – Urine was collected in toluene by keeping the animals in metabolic cages. At the end of the collection period, the samples were alkalinized with a borate buffer, 1 M, pH 10.5 and extracted with ethyl ether. The residue obtained after low pressure evaporation at 40 °C was redissolved in methanol and quantitatively applied to a silica-gel thin layer chromatographic plate together with a reference sample of the pure radioactive drug. After elution with a system containing n-butanol, ammonium hydroxide and water (8:1:1) and visualization with iodine vapors, the corresponding spot was scraped off the plate and submitted to liquid scintillation counting (LSC).

Alternatively, direct determinations of SH groups or of SH plus S-S groups were performed by Ellman's method (1958) or by Habeeb's method (1973), respectively.

b) *Total radioactivity assay:* for this part of the experiment, ^{35}S-t-BAESS, with a specific activity of 388 dpm/μg was used.

Samples of blood plasma (0.1-0.2 ml) or tissue homogenates (50 μg) were treated with 0.6 M sodium hydroxide and heated at 80 °C in a sand bath until completely digested. After evaporation to obtain a clear residue, 4% hydrochloric was added and subsequently neutralized with ammonium hydroxide.

In the next step a modified Bray cocktail was added and the activity counted for 10 min in a scintillation counter. Alternatively, for total radioactivity assay of urine, samples were simply counted by LSC after addition of the Bray cocktail.

c) *Pharmacokinetic parameters:* these parameters were calculated according to the methods described in the specialized literature.

The $AUC_{I.V.}$ (area under the intravenous curve) was calculated by a computer program BMDP-3R. The AUC_{oral} was calculated by the trapezoidal method.

RESULTS

Results obtained with a 35 mg/kg – dose: as shown in Fig. 1, the blood levels for both total and free drug followed a linear decay after an I.V. injection of the drug and, therefore, the kinetics seem to obey the one open compartment model, although for the total drug there is some indication of a short distribution phase, possibly related to a retention of the drug molecules by the blood components during the first few minutes.

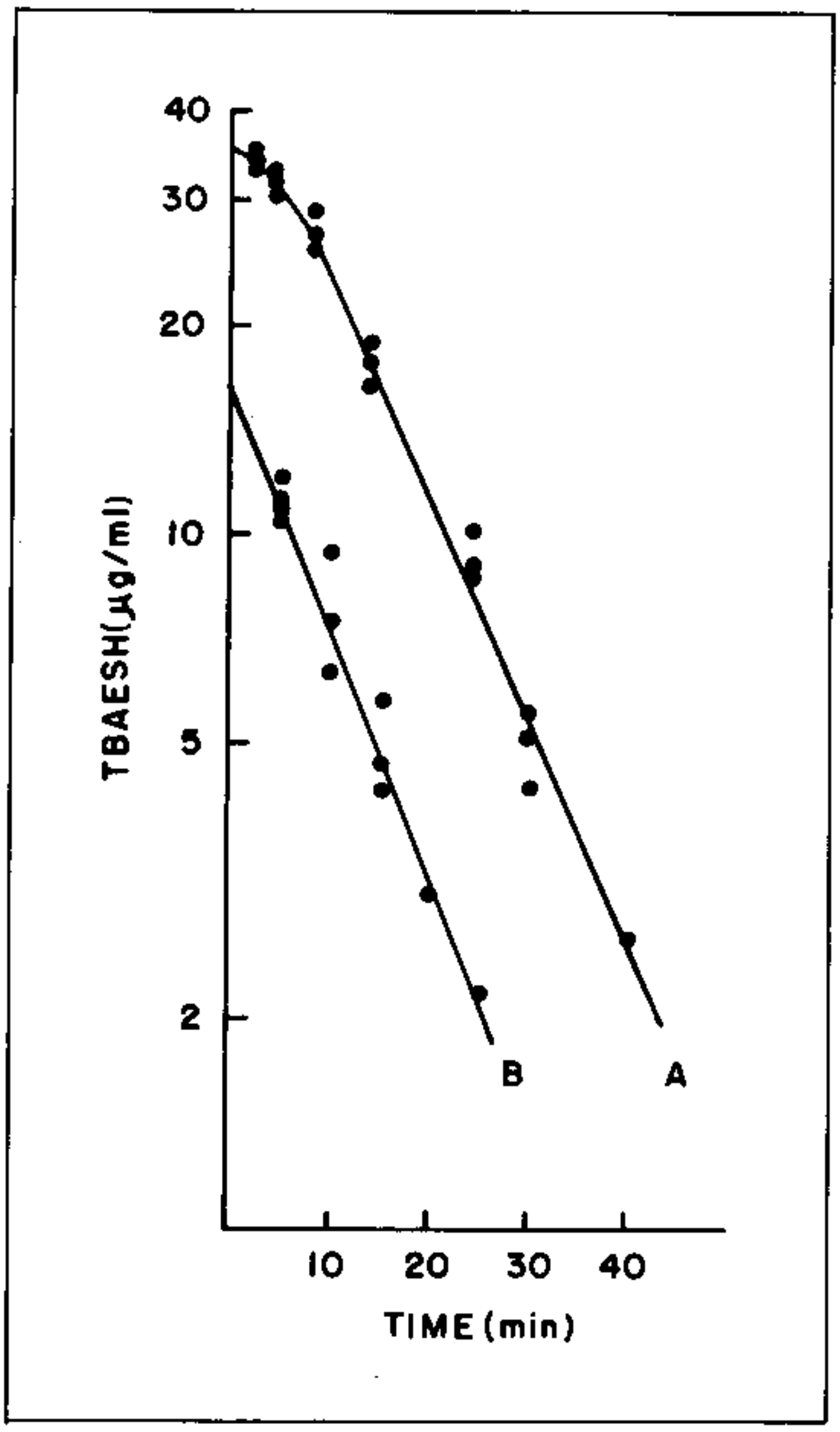

Fig. 1: blood levels of unaltered drug measured as TBAESH after intravenous administration of TBAESS (35 mg/kg). Curve A refers to total (free + bound) drug. Curve B refers to free drug. Each point represents a pool from 3-6 animals (for concentrations above 5 μg/ml, 3 animals were used; for lower concentrations, 6 animals).

From these data some pharmacokinetic parameters were calculated and are shown in Table I. According to this table, the AUC for the total drug was about 3.8 times larger than that for the free drug, the rate constant of elimination was a little (1.17 times) larger for the free drug and, consequently, the biological half life (T½) is about 1.17 times longer for the total than for the free drug. On the other hand, the distribution volume for the free drug was about 2.73 times larger than that for total drug.

The blood levels of the total drug, after oral administration, are depicted in Fig. 2. It can be verified that the absorption from the gastrointestinal tract was relatively rapid, with a peak level at about 25 min. There were no detectable amounts of drug in the blood beyond 60 min after administration.

TABLE I

Pharmacokinetic parameters calculated for total and free TBAESS in blood after a single intravenous administration (35 mg/kg)

Parameters*	Total drug	Free drug
AUC ($\mu g.min.ml^{-1}$)	671 ± 14.0	209 ± 10
Kel (min^{-1})	0.071 ± 0.0034	0.083 ± 0.0082
T½ (min)	9.8 ± 0.47	8.4 ± 0.81
Vd/g (ml/g)	0.74 ± 0.055	2.02 ± 0.29

AUC – Area under the curve of blood drug concentration versus time
Kel – Elimination rate constant
T½ – Biological half-life
Vd/g – Specific distribution volume.

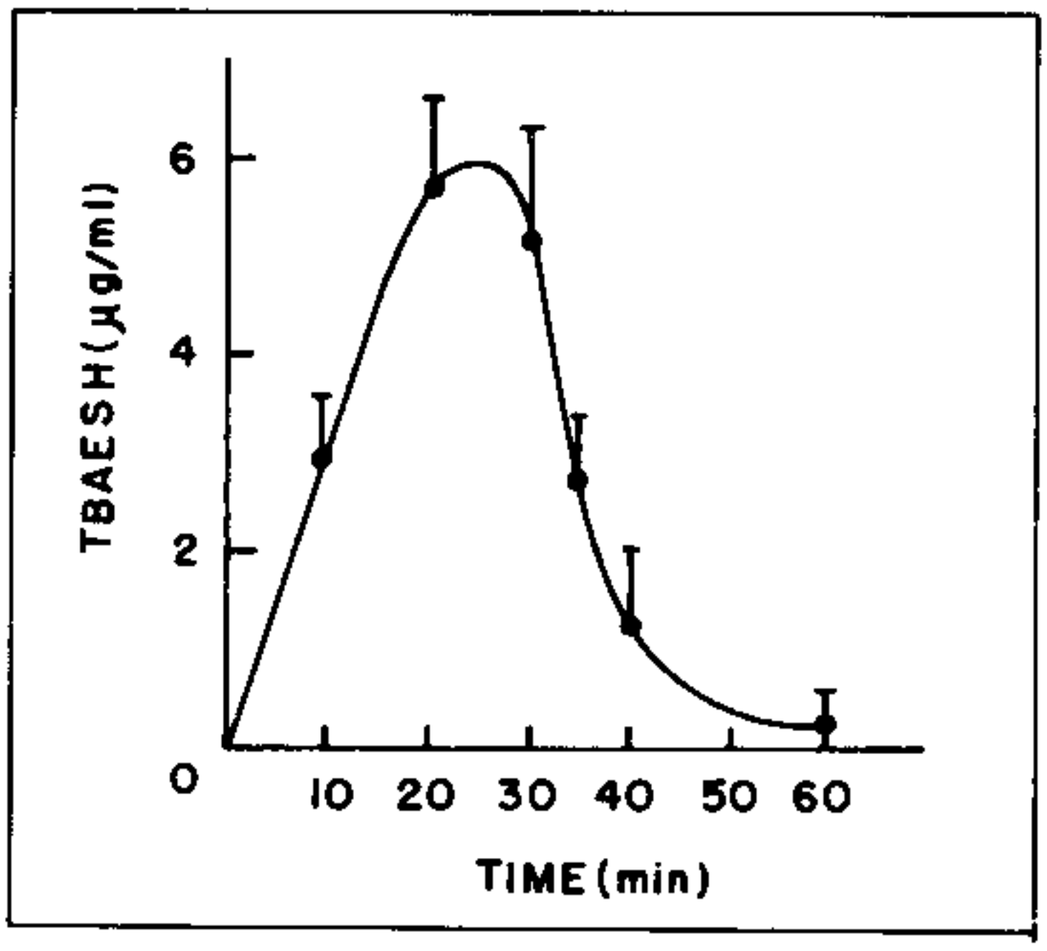

Fig. 2: blood levels of unaltered total drug measured as TBAESH after oral administration of TBAESS (35 mg/kg). Each point represents the average of three determinations ± standard deviation (S.D.).

TBAESS biodisponibility was 0.22 ± 0.05, a value calculated from the ratio of AUC_{oral} (150 ± 40) to $AUC_{I.V.}$ (671 ± 14 μg.min.ml^{-1}). This parameter represents the fraction of unaltered drug reaching the systemic circulation after passing through the liver.

TBAESS concentrations (μg/ml) in the blood and its several components at different time intervals after drug administration are shown in Table II. It can be seen that, at all the time intervals, the protein bound drug percentages were higher than those of the other two blood components.

TABLE II

TBAESS concentrations in blood and its main components after a single intravenous administration (35 mg/kg)

Time intervals (min)	TBAESS concentrations (μg/ml of blood)			
	Whole blood	Blood cells	Plasma	
			Free forms*	Bound forms**
5	31.5	9.2	10.9	11.4
10	24.1	5.9	7.1	11.4
15	16.2	4.1	4.8	7.3

* Plasma drug concentration not bound by S-S bridge.
** Obtained by difference: drug bound by S-S bridge.

Fig. 3 depicts intestinal absorption of the drug. It may be seen that about 50% of the drug was rapidly absorbed in the first 20 min, and the remainder a little more slowly, until complete at about 120 min.

Results obtained with a 300 mg/kg dose: after oral administration of ^{35}S labeled drug, a peak level of total plasmatic radioactivity (representing drug plus metabolites) was reached at about 180 min (Fig. 4). A slow monoexponential decrease occurred thereafter, in accordance with a one-compartment pharmacokinetic model, and the T½ for this decay was 5.9 h.

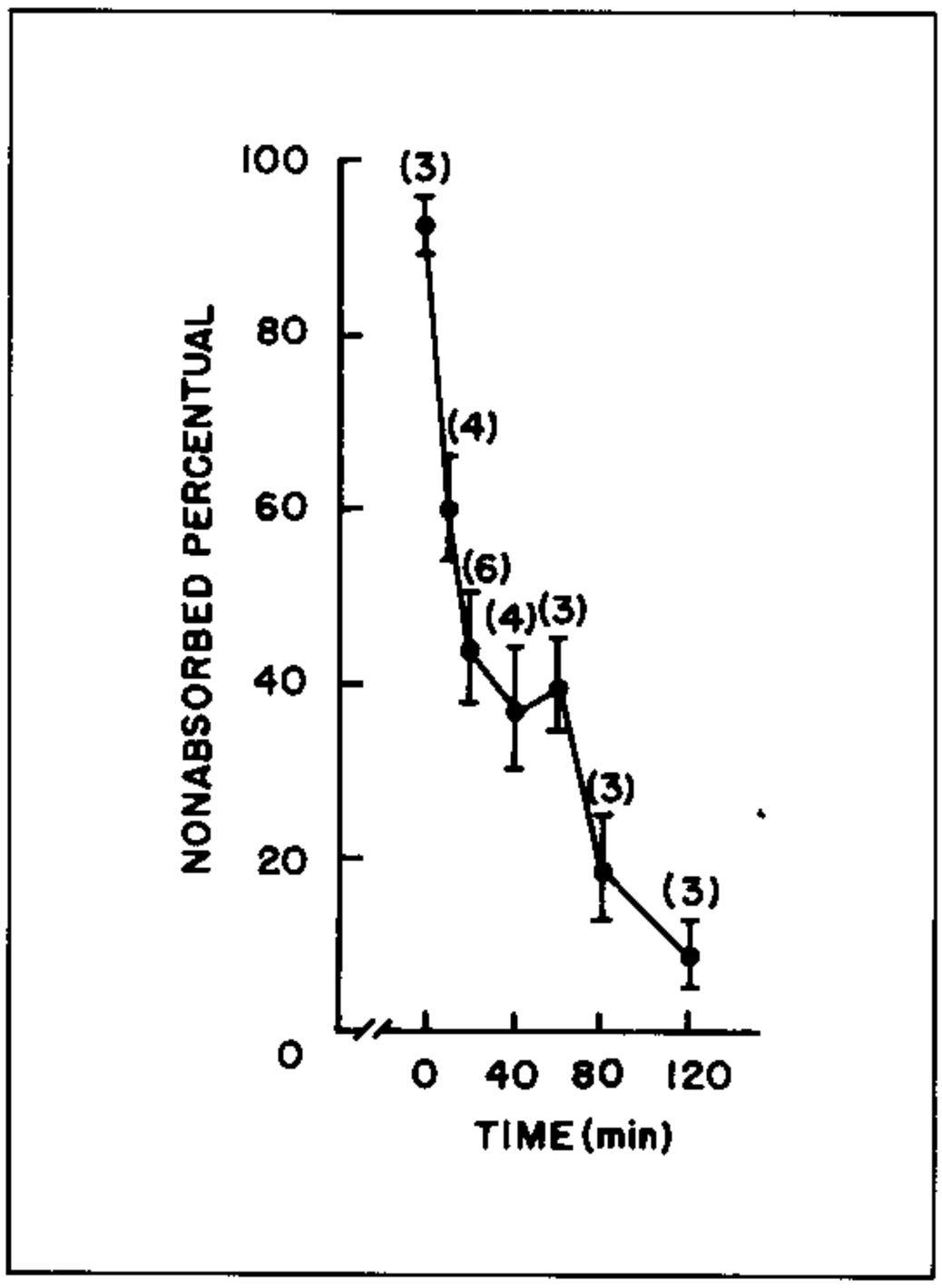

Fig. 3: nonabsorbed drug after oral administration of TBAESS (35 mg/kg). Each point represents the average ± S.D. Parenthesis refer to the number of determinations.

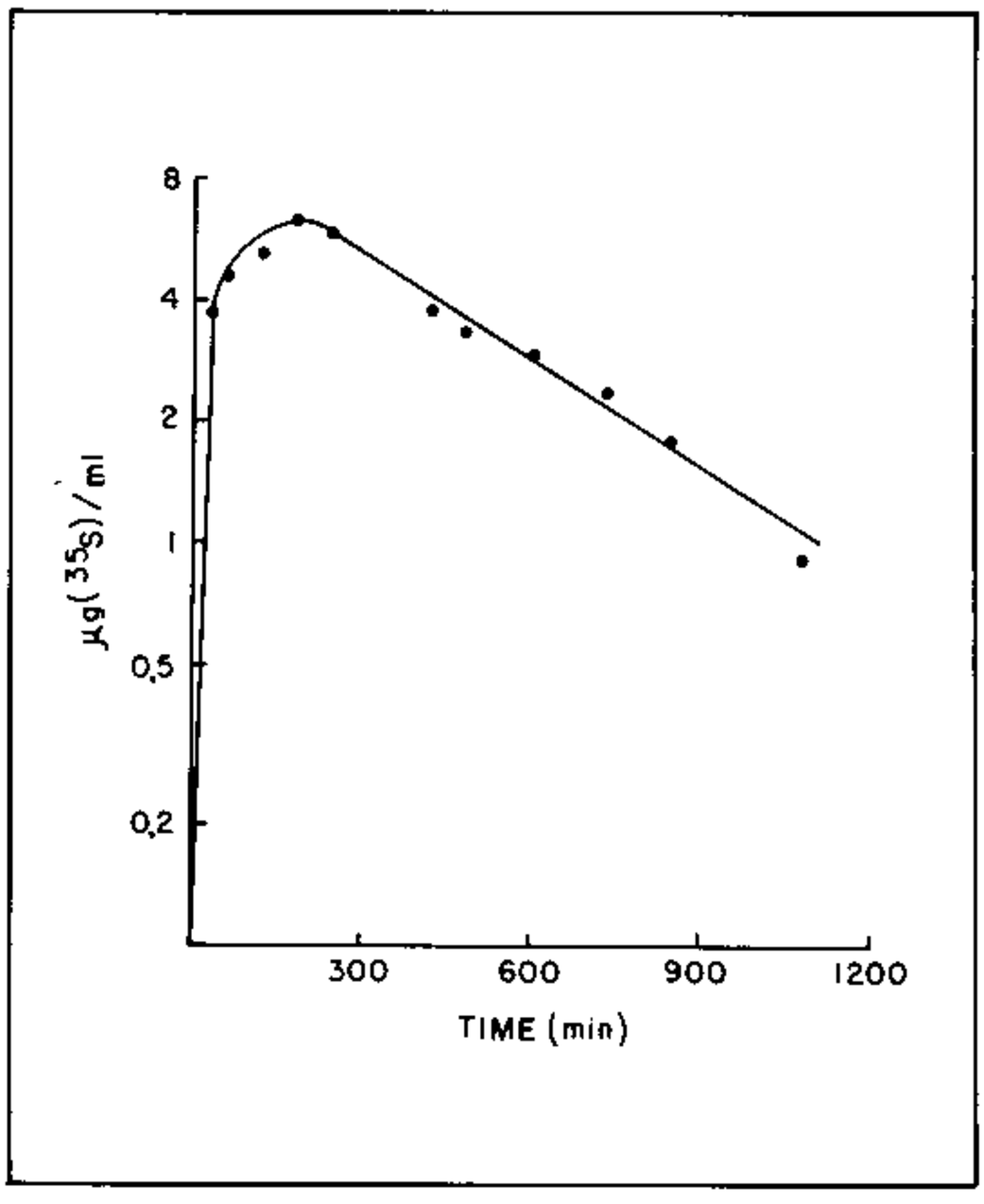

Fig. 4: semi-log graph showing plasmatic ^{35}S decay after oral administration of $TBAESS^{35}$ (300 mg/kg). Each point represents the average of 3 determinations.

The urinary excretion of total radioactivity was also studied in these animals. It was verified that in 6 h only about 13% of the administered radioactivity appeared in the urine. On the other hand, Fig. 5 shows that the percent excreted increased gradually until reaching a value close to 60% in 25 h. At this time, although about 40% of the initial amount administered was still remaining in the organism, the ^{35}S appearing in the feces was about 4% of the initial amount.

At the same time, as indicated in Table III, a small percentage (4.8%) of unmetabolized drug was excreted in the urine 26 h after its administration. An analysis of the total radioactivity remaining in several tissues after 18 and 26 h, respectively, is shown in Fig. 6.

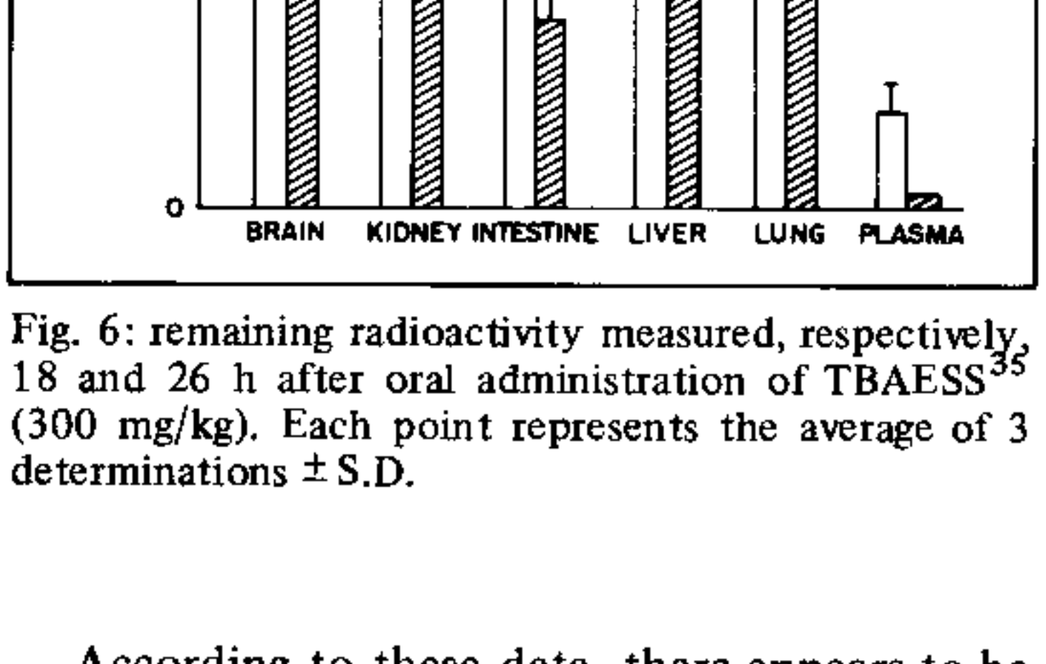

Fig. 6: remaining radioactivity measured, respectively, 18 and 26 h after oral administration of TBAESS^{35} (300 mg/kg). Each point represents the average of 3 determinations ± S.D.

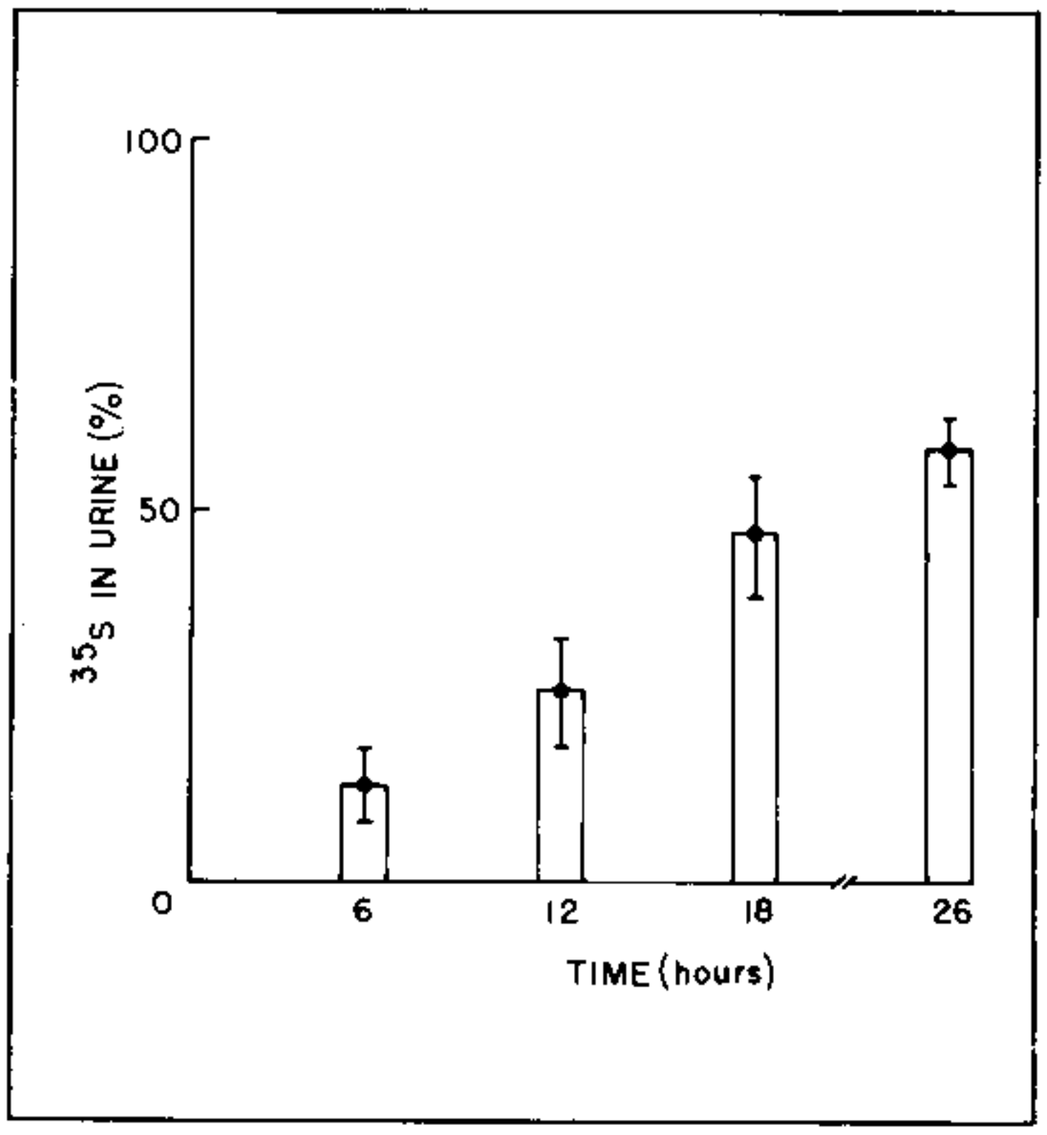

Fig. 5: ^{35}S cumulatively excreted in urine after administration of TBAESS^{35} (300 mg/kg – orally). Three determinations ± S.D. for each point.

According to these data, there appears to be an intense drug metabolization just after its oral administration. Actually, the data in Table IV suggest that most of the drug was metabolized in the first six hours after the administration, since no more than about 3% of unaltered drug is excreted in 6, 12 and 18 h after oral administration of 300 mg/kg.

The pattern for blood TBAESS levels after a single oral administration of a high dose is shown in Fig. 7. For the total drug, a peak level (29.7 μg/ml) at about 90 min, followed by a period of oscillating levels, and then a slow even decrease beginning at about 240 min were observed. For the free drug, a peak level of only about 4 μg/ml was observed about 35 min after administration, followed by an even decrease in the blood levels.

TABLE III

TBAESS excreted in urine 26 h after oral administration (300 mg/kg – single dose)*

Excretion form	Percentage excreted
SH	1.6 ± 0.46
SH ± SS	4.8 ± 1.14

* Average of three experiments ± S.D.
SH: thiol form; SS: disulfide form.

TABLE IV

TBAESS^{35} excreted in urine after a single administration of the labelled drug (300 mg/kg)

Time elapsed after administration (hours)	Percentage excreted as TBAESS^{35} ± S.D.
6	2.95 ± 0.62
12	2.35 ± 0.53
18	2.59 ± 0.81

Fig. 8 depicts intestinal absorption of the drug after administration of a 300 mg/kg-dose and shows that about 50% was absorbed in the first 40 min, most of the drug remaining in the lumen for a long time with incomplete absorption even at the end of 320 min.

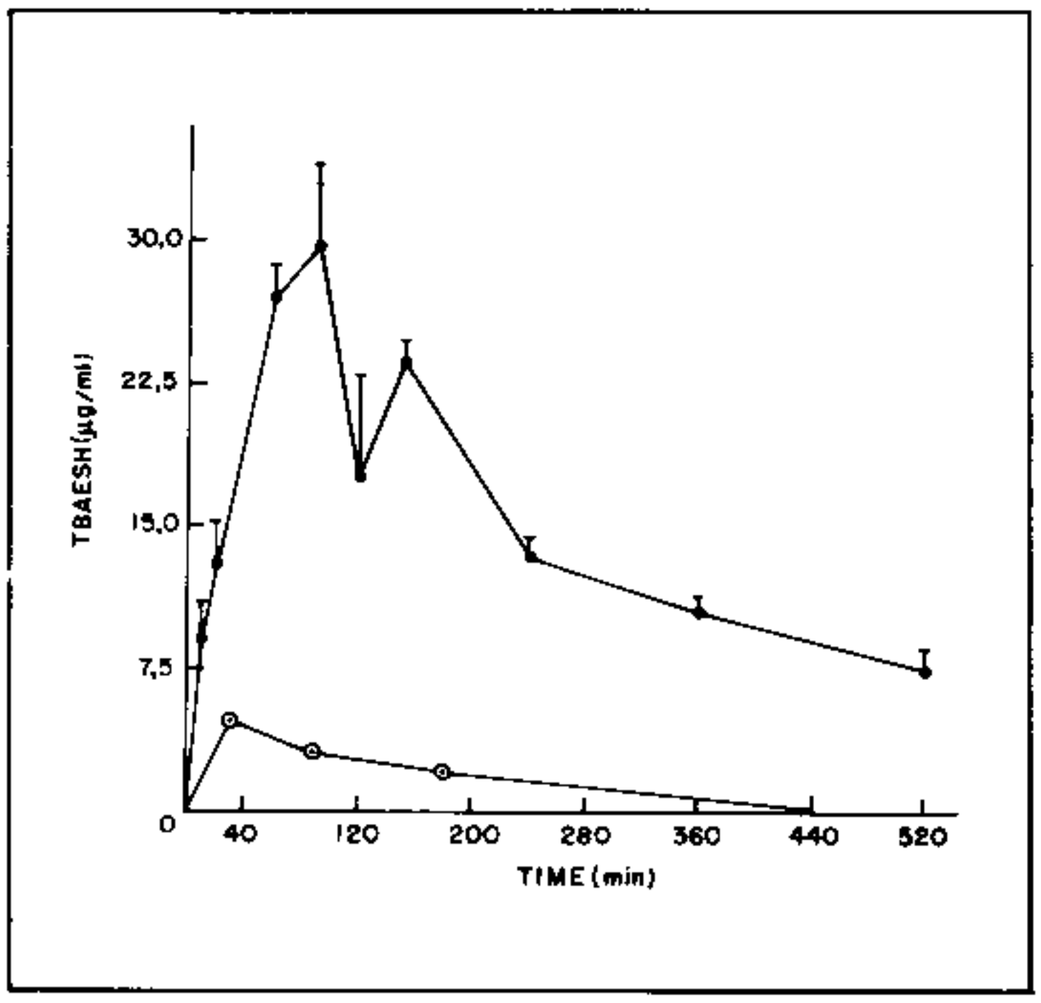

Fig. 7: blood levels of unaltered drug measured as TBAESH after oral administration of TBAESS (300 mg/kg). Three determinations for each point. ○–: Free drug ●–: Total (free + bound) drug.

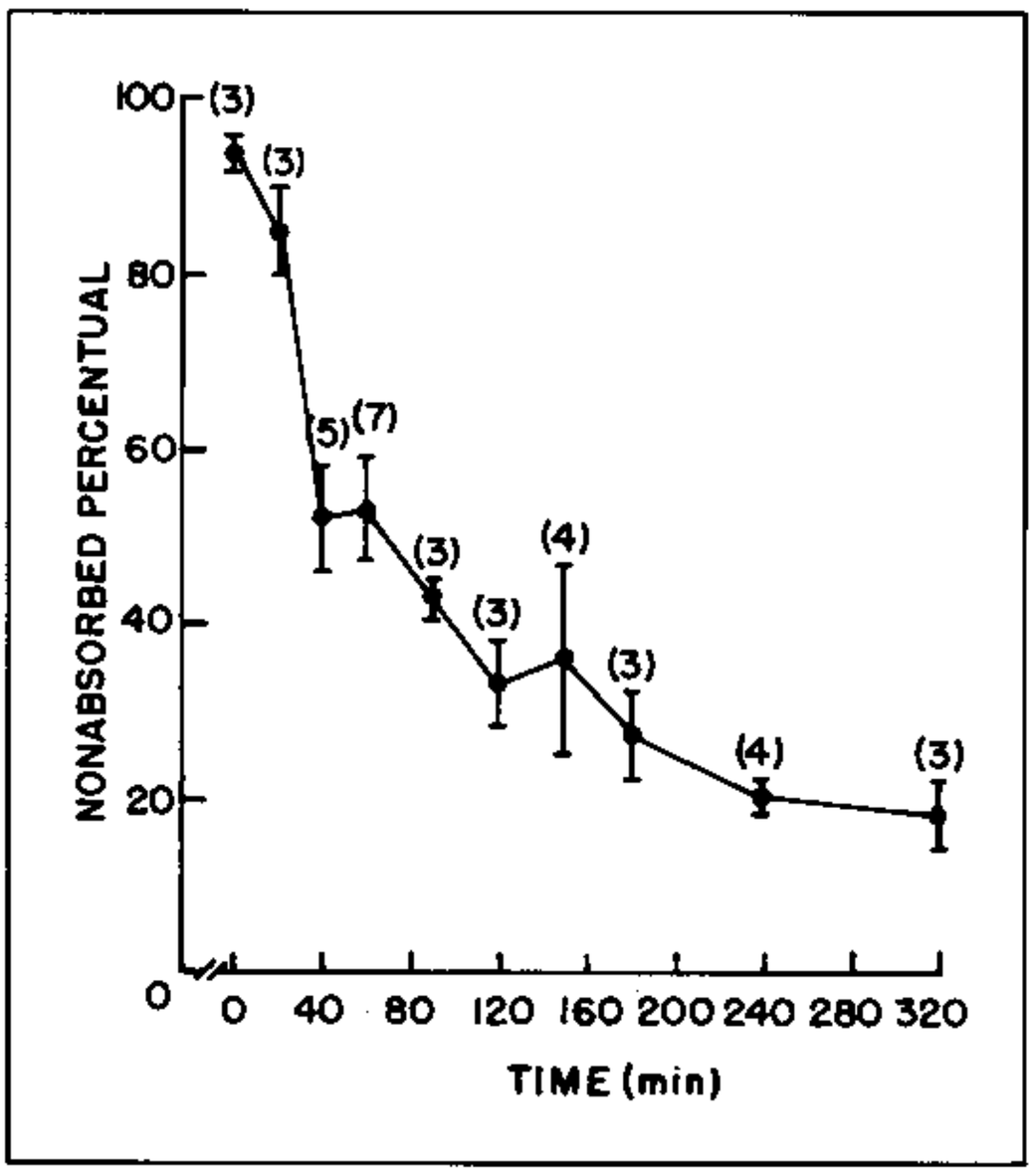

Fig. 8: nonabsorbed drug after oral administration of TBAESS (300 mg/kg). Each point represents the average ± S.D. Parenthesis refer to the number of determinations.

DISCUSSION

TBAESS seemed to follow a dose-dependent pharmacokinetic behavior in such a way that when administered in a 35 mg/kg-dose, it usually obeyed the one open compartment model, and, when administered in a 300 mg/kg-dose, a much more complex model is apparent.

In the latter situation, saturation of the tissues appears to occur, resulting in periods of oscillation in the levels of unmetabolized drug which are probably due to some type of momentary drug displacement from these tissue sites (Fig. 7). In other words, some tissue-blood drug interchange seems to occur in such a way that at a certain moment the drug displaces from tissues, enters the blood and returns to the tissues in the next step. A similar behavior was observed by Pedersen (1980) with disulfiram, a drug which also displays SH groups in its structure.

The T½ for total radioactivity as compared to that for unmetabolized drug indicated that the radioactivity (drug plus metabolites) was slowly excreted. This fact could be due to tissue binding of ^{35}S compounds, which would decrease the amount of drug or of drug-derived substances available for excretion. Such a behavior was also noticed for mercaptoethylamine, a substance with close structural similarities to TBAESS (Aebi, 1957). On the other hand, the data regarding tissue radioactivity levels (Fig. 6) and urinary excretion (Fig. 5) indicated that at 26 h, when the plasma radioactivity was very low, there was still a considerable amount (around 60% of the total administered) of ^{35}S in the organism.

In this regard, our findings are also similar to those obtained by Verly et al. (1954), who observed that after 24 h the amount of cysteamine present in the mouse body was negligible, although 34% of the injected radioactivity was still retained in the organism.

The T½ for the unmetabolized drug indicated that it was rapidly eliminated from the organism. There were some hints that the main elimination process for this drug was possibly metabolization. One of these hints was the biodisponibility value: it indicated that about 78% of the drug was metabolized upon the first passage through the liver. Another indication for such an occurrence was the fact that after

nearly 6 h from the time of administration, the excretion of unaltered drug became practically constant (Table IV), although the total radioactivity in the urine increased until at least 26 h (Fig. 5).

On the other hand, an accentuated difference in the animals' reactions when the drug was administered by I.V. and oral routes was noticed (personal observations). When administered I.V. the maximum tolerated dose seemed to be about 35 mg/kg, whereas the animals could receive up to 300 mg/kg orally without toxicological manifestations. This fact should mean that, when administered orally, the drug is rapidly metabolized in the liver and, consequently, does not reach the systemic circulation in its unmodified and probably more toxic form.

Some doubts about the effectiveness of this drug against schistosomiasis would be manifested upon consideration of such a high metabolization rate. However, two facts should be taken into consideration: the first is that the parasites are localized mainly in the portal system and, therefore, are subjected to the drug action before it is metabolized. The second fact is that, although not yet confirmed, there is the possibility of some of the drug metabolites being active against the parasites so that, even after passing through the liver some activity may still be present.

The drug absorption from the G.I. tract seemed to be mediated by some type of active or facilitated process that could be saturated at high doses. Figs 2, 3, 7 and 8 provided the main elements for such a hypothesis. With the 35 mg/kg-dose (Figs 2, 3), the intestinal absorption was relatively rapid, being complete in 120 min; the peak blood levels were rapidly attained in about 25 min. With the 300 mg/kg-dose, the peak blood level was attained only in about 90 min after administration and the absorption was not complete even 320 min thereafter.

Considering that it is the free drug which is able to reach the tissues and, therefore, may be responsible for toxicity, it is possible to say that the maximum tolerated free drug blood-level seems to be approximately 16 μg/ml. Such an affirmation is made possible by analyzing Figs 1 and 7, respectively. Fig. 1 shows that the maximum blood level of free drug attained after I.V. injection of a 35 mg/kg-dose is around 16 μg/ml, and our observations indicated that in this situation no major toxicity signals were observed. Whenever higher doses were injected, the animals died. On the other hand, results depicted in Fig. 7 indicate that the maximal blood level of free drug after oral administration of a 300 mg/kg-dose was about 5 μg/ml. Such a level was never toxic.

In summary, these results suggest that the differences in drug behavior observed when it was administered by different routes may be linked to at least two of the factors listed in the introduction of this paper: a) the high rate of hepatic metabolism and b) the increased drug distribution.

RESUMO

Aspectos farmacocinéticos do t-butilamino-etil-dissulfeto. Uma droga experimental na esquistossomose em camundongos – Um estudo preliminar da farmacocinética do t-butilamino-etil-dissulfeto foi conduzido utilizando droga fria ou radioativa em duas diferentes doses (35 e 300 mg/kg).

Amostras de plasma ou sangue foram tratàdas com ditiotreitol, ácido perclórico, e, após filtração, submetidas a uma subseqüente purificação em um "batch" de resina aniônica. Na etapa final, a droga foi retida em coluna de resina catiônica, eluída com NaCl 1 M e detectada pelo método de Ellman (1958). Alternativamente, a droga radioativa foi detectada por cintilação líquida.

Os resultados correspondentes à droga total administrada na menor dose sugeriram um comportamento farmacocinético relacionado ao modelo de um compartimento aberto, com os seguintes parâmetros: área sob a curva intravenosa ($ASC_{I.V.}$): 671 ± 14; ASC_{oral}: 150 ± 40 $\mu g.min.ml^{-1}$; constante de eliminação: 0,071 min^{-1}; meia-vida biológica: 9,8 min; volume de distribuição: 0,74 ml/g.

Para a dose mais alta, os resultados indicaram aparentemente a ocorrência de um modelo mais complexo e não adequadamente classificado. Analisados em conjunto os resultados sugerem a ocorrência de um comportamento farmacocinético dose-dependente. A droga é absorvida e eliminada rapidamente, sendo este último

processo relacionado principalmente à metabolização.

A droga parece mais tóxica quando administrada via I.V. porque por esta via ela não sofre metabolismo de primeira passagem e é, por outro lado rapidamente distribuída para os tecidos. O nível sanguíneo máximo tolerado pelos animais parece ser de 16 μg/ml.

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