

PHARMACOKINETIC PROFILE OF TERT-BUTYLAMINOETHANETHIOL

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A preliminary study of the pharmacokinetic parameters of t-Butylaminoethanethiol (TBAESH) was performed after administration of a single dose (35 mg/kg) either orally or intravenously.

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).

The results suggested a pharmacokinetic behavior related to a one open compartment model with the following values for the total drug: area under the intravenous curve ($AUC_{i.v.}$): 443 ± 24.0 ; AUC_{oral} : $85.5 \pm 14.5 \mu\text{g min.ml}^{-1}$; elimination rate constant: $0.069 \pm 0.0055 \text{ min}^{-1}$, biological half-life: $10.0 \pm 0.80 \text{ min}$; distribution volume $1.15 \pm 0.15 \text{ ml/g}$; bioavailability: 0.19 ± 0.02 . From a pharmacokinetic standpoint, TBAESH seems to have no advantage over the analogous disulfide compound.

Key word: aminoethyl pharmacokinetics

In a preceding paper (Guerra Andrade et al., 1989), some pharmacokinetic aspects of tert-butylaminoethyl disulfide (TBAESS) were discussed. According to Nelson & Pellegrino (1976), tert-butylaminoethanethiol (TBAESH) is also active against *Schistosoma mansoni*, so a study of its pharmacokinetics was performed in order to appreciate the possible influence of a structural modification on the drug disposition in the mouse organism.

Simultaneously, this study may contribute some insight which would permit the selection of similar members of this group of drugs studied by the last named authors, so as to maximize the activity against the parasite.

MATERIALS AND METHODS

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

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

Drugs and reagents – TBAESH was prepared from t-butylaminoethanol (Aldrich) by classical methods via the intermediate aminobromide (Blatt, 1943) and isothiuronium salt (Vogel, 1956) (m. p. of the hydrochloride salt = $187-190^\circ\text{C}$). The other substances used were obtained as described by Guerra Andrade et al. (1989).

Isolation and detection of the unaltered drug

From blood samples: total drug (free plus bound) – After decapitation of the animals at regular intervals, blood samples were immediately taken, treated with dithiothreitol (DTT) 0.6 M, submitted to protein precipitation with perchloric acid (HClO_4) at a final concentration of 0.4 M and centrifuged at 21,000g 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 1 h and the final step of the isolation process involved retention of the

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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 transferred to tubes containing HClO_4 and the samples purified as described above for the total drug.

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 centrifuged (21,000g, -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.

Pharmacokinetic parameters – These parameters were calculated according to the methods described in the specialized literature. The $\text{AUC}_{\text{I.V.}}$ (area under the intravenous curve) was calculated by a computer program BMDP-3R. The AUC_{oral} was calculated by the trapezoidal method.

RESULTS

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.

From these data some pharmacokinetic parameters were calculated and these are shown in Table I. According to this table, the AUC for the total drug was about 2.4 times greater than that for the free drug while the rate constant of elimination (K_{el}), as well as the biological half-life ($T_{1/2}$), were about the same for both free and total drug. On the other hand, the distribution volume (V_d) for the free drug was about 2.2 times larger than that for the 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 gastro-intestinal (G.I.) tract was relatively rapid, with a peak level at about 10 min. There were no detectable amounts of drug in the blood beyond 40 min after administration.

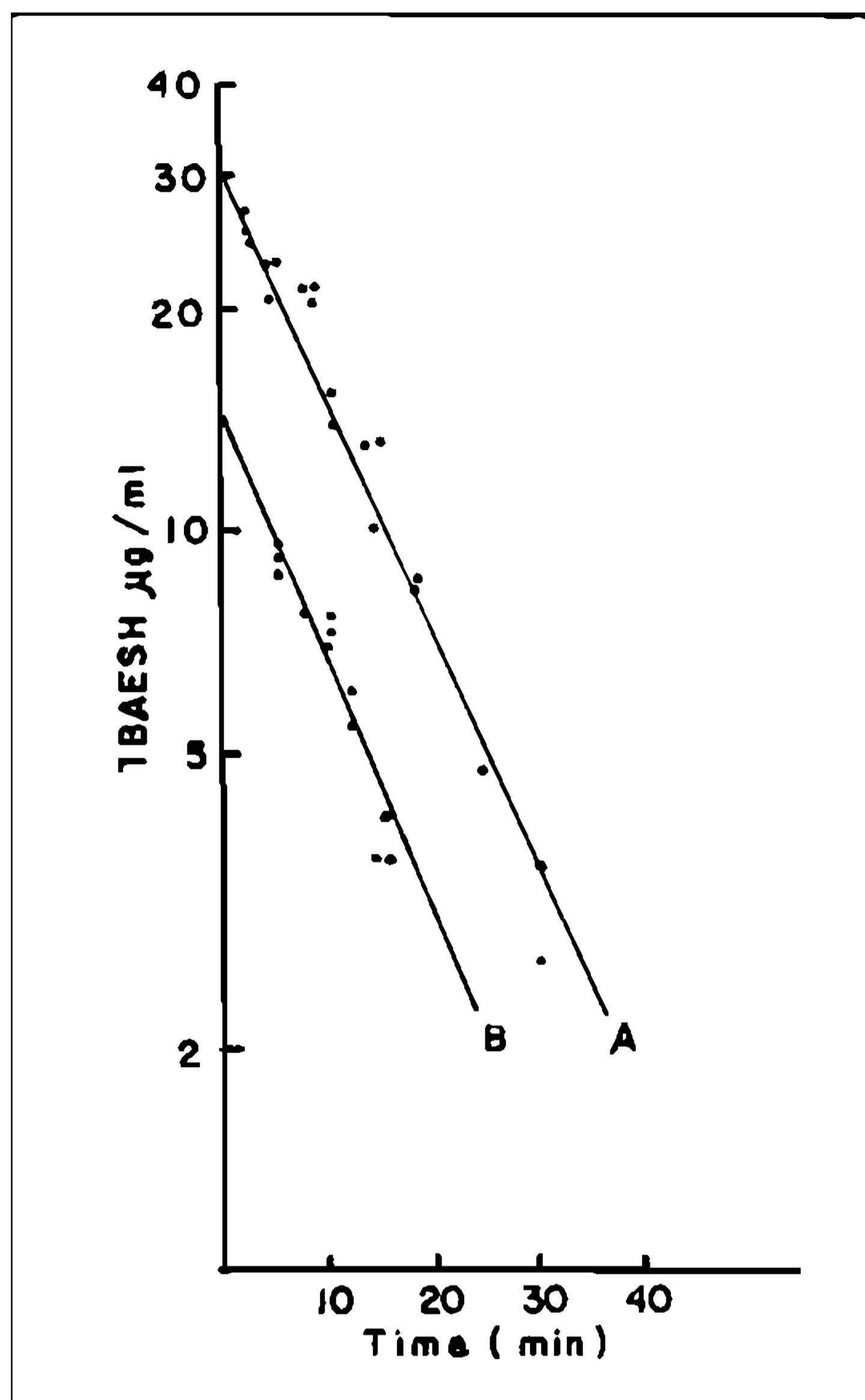


Fig. 1: blood levels of unaltered drug after intravenous administration of TBAESH (35 mg/kg). Curve A refers to total (free + bound) drug. Curve B refers to free drug. Each point represents a pool from 3-6 animals (for concentrations above $5 \mu\text{g/ml}$ 3 animals were used; for the lower concentrations 6 animals).

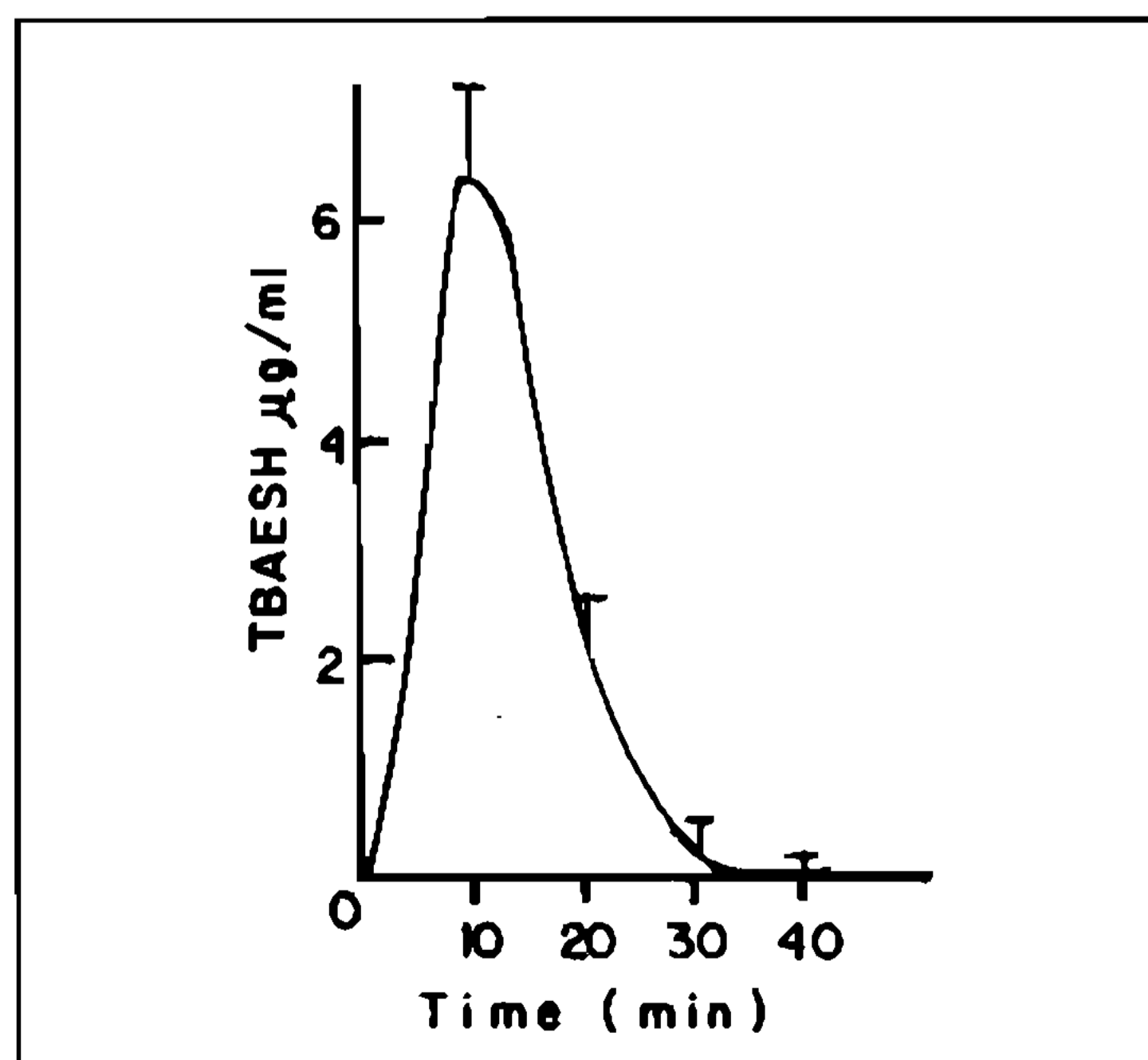


Fig. 2: blood levels of unaltered total drug after oral administration of TBAESH (35 mg/kg). Each point represents the average of 3 determinations \pm standard deviation (S. D.).

TABLE I

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

Parameters	Total drug	Free drug
AUC (mg.min.ml ⁻¹)	443 ± 24.0	184 ± 9.4
Kel (min ⁻¹)	0.069 ± 0.0055	0.074 ± 0.0081
T 1/2 (min)	10 ± 0.80	9.43 ± 1.03
Vd/g (ml/g)	1.15 ± 0.15	2.60 ± 0.42

AUC – Area under the curve of blood drug concentration versus time; Kel – Elimination rate constant; T 1/2 – Biological half-life; Vd/g – Specific distribution volume.

TABLE II

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

Time intervals (min)	TBAESH concentrations (µg/ml of blood)			
	Whole blood	Blood cells	Plasma	
			Free forms ^a	Bound forms ^b
5	21.0	7.7 (37)	9.8 (46)	3.5 (17)
10	14.5	4.6 (32)	6.6 (45)	3.3 (23)
15	10.2	3.0 (30)	4.5 (44)	2.7 (26)

^aPlasma drug concentration not bound by S-S bridge.

^bObtained by difference: drug bound by S-S bridge.

Numbers in parenthesis represent the respective percentages of drug found in blood components, considering the figures for "whole blood" as 100%.

TBAESH biodisponibility was 0.19 ± 0.02 , a value calculated from the ratio of AUC_{oral} (85.5 ± 14.5) to $AUC_{\text{i.v.}}$ (443 ± 24.0 µg.min.ml⁻¹); this parameter representing the fraction of unaltered drug reaching the systemic circulation after passing through the liver.

TBAESH 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 values for plasma free drug were higher than those for the bound forms (an average of 45% as free forms and 22% as the bound ones, from 5 to 15 min after administration).

Figure 3 depicts intestinal absorption of the drug. It may be seen that about 65% of the drug was rapidly absorbed in the first 10 min, and the remainder a little more slowly during the following 60 min.

DISCUSSION

TBAESH, when administered in a 35 mg/kg dose, showed a pharmacokinetic profile similar to that observed for its disulfide derivative, as described in a preceding paper (Guerra Andrade et al., 1989).

Some differences between the two compounds should otherwise be emphasized. For

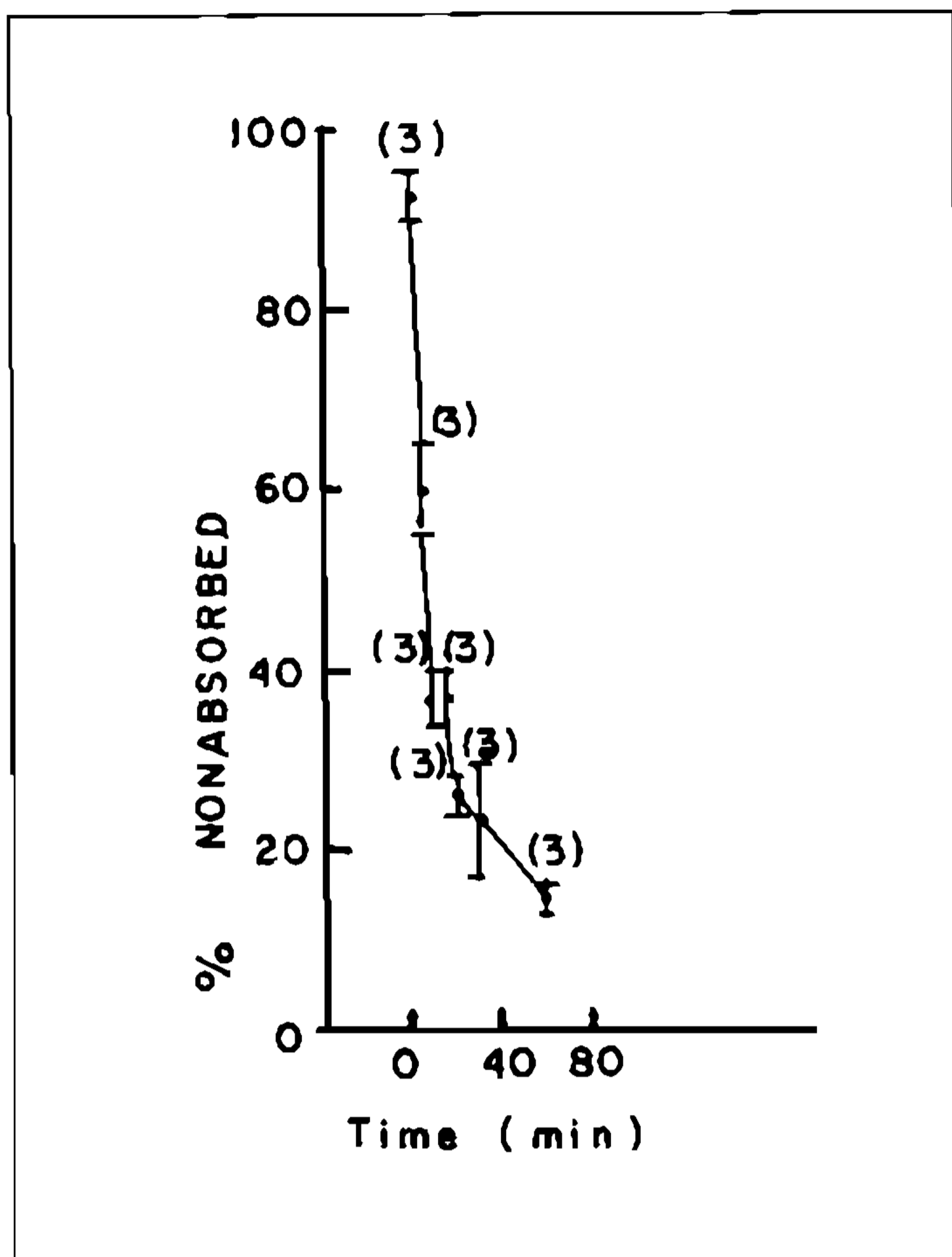


Fig. 3: nonabsorbed drug after oral administration of TBAESH (35 mg/kg). Each point represents the average \pm S. D. Parenthesis refer to the number of determinations.

example, the profile of total-drug blood level decay for TBAESH did not show the short distribution phase observed for TBAESS, probably because TBAESH is less extensively retained by the blood components than the disulfide.

The AUC value for either total or free TBAESH was smaller than the same values for TBAESS. As a consequence the distribution volumes for the thiol compound were higher

than those for the disulfide. Another factor contributing to a higher V_d value for TBAESH as compared to TBAESS is the fact that almost twice as much disulfide is bound to blood plasma than the thiol compound (respectively 42 and 22% on the average, from 5 to 15 min after administration). Altogether, these facts should mean that TBAESH is a drug with a greater affinity for tissues than TBAESS.

Observed values for $T_{1/2}$, K_{el} and bio-disponibility were not significantly different for the two drugs. The drug absorption from the G. I. tract, as in the case of TBAESS, seemed to be mediated by some type of active or facilitated process and was faster than that observed for the latter drug.

In summary, the results indicate that, from a pharmacokinetic standpoint, TBAESH should not have any pronounced advantages over TBAESS.

REFERENCES

- BLATT, A. H., 1943. *Organic Synthesis*, Coll. Vol. II. 1st ed. Wiley. New York, p. 91.
- ELLMAN, G. L., 1958. A colorimetric method for determining low concentrations of mercaptans. *Arch. Biochem. Biophys.*, 74: 443-450.
- GUERRA ANDRADE, M. H.; FREIRE, A. C. T. & NELSON, D. L., 1989. Pharmacokinetic aspects of tert-Butylaminoethyl disulfide, an experimental drug against schistosomiasis in mice. *Mem. Inst. Oswaldo Cruz*, 84: 95-102.
- NELSON, D. L. & PELLEGRINO, J., 1976. Experimental chemotherapy of schistosomiasis mansoni. XIV. Active derivatives of aminoethanephosphorothioate, disulfide and isothioureia. *Rev. Inst. Medic. Trop. São Paulo*, 18: 443-449.
- VOGEL, A. I., 1956. *A Textbook of Practical Organic Chemistry*. 3rd. ed. John Wiley & Sons - New York. p. 277 and 497.