Table II—Blood Physostigmine Levels ($\times 10^{-7}$ *M*) in Humans 30 min after Administration

Route	Dose Administered, μg/kg				
	15	20	25	30	45
Intravenous	—[a]			0.40	0.90
	—[a]			0.29	0.59
	—[a]				0.90
	—[a]				0.78
Intramuscular	—[a]	0.35	0.25		
	—[a]	—[a]	0.31		
		—[a]	0.27		
			—[a]		

[a] $<0.25 \times 10^{-7}$ *M*.

of data obtained from a reproducibility study. Ten whole blood samples were prepared at each of six different concentrations of physostigmine, ranging from 0.25 to 5.00 $\times 10^{-7}$ *M*. Means, standard deviations, and coefficients of variation for each set of 10 assays are presented in Table I. Precision was within ±3.8% for physostigmine concentrations ranging from 0.50 to 5.00 $\times 10^{-7}$ *M*. At 0.25 $\times 10^{-7}$ *M*, precision was ±10.0%.

The mean physostigmine concentrations obtained in the precision study were correlated with the concentrations added to the blood samples. The plot of data points and the equation of the regression line are given in Fig. 3.

Blood levels at 30 min after intravenous and intramuscular injections of physostigmine are presented in Table II. The relationship of dose administered to blood levels was approximately the same for both routes of administration.

DISCUSSION

To relate physostigmine concentration to cholinesterase inhibition, it is necessary to measure enzyme activity after inhibition has progressed until none of the enzyme remains in the complexed form. At this time, when the enzyme activity is between 30 and 70% of the control level, only decarbamylation occurs. The time course at 37°, from inhibition until a near linear reactivation rate is attained, together with the enzyme activity level at this time, can be used to determine the concentration of physostigmine in the blood at the time the *in vitro* sample was inhibited. With an *in vivo* blood sample, the physostigmine concentration can be determined for the time the blood was withdrawn from the patient.

This assay method assumes that decarbamylation occurs over the entire period from the time of inhibition. The error in the assumption is quite small, since an initial time period of less than 2 min is required for formation of an appreciable concentration of carbamylated enzyme. The time required for enzyme reactivation should be, and has been shown to be, a function of the inhibitor concentration. The time required to complete an assay varies directly with the physostigmine concentration, from approximately 1 hr for 0.50 $\times 10^{-7}$ *M* to approximately 7.5 hr for 5.00 $\times 10^{-7}$ *M*. Considering the inherent errors, the precision of this method is excellent.

REFERENCES

(1) F. H. Rodin, *Am. J. Ophthalmol.*, **30**, 19 (1947).

(2) M. D. Walker, *Lancet*, **1**, 1200 (1934).

(3) G. R. Farrer and J. J. Miller, *Am. J. Psychiat.*, **115**, 455 (1958).

(4) E. B. Crowell, Jr., and J. S. Ketchum, *Clin. Pharm. Ther.*, **8**, 409 (1967).

(5) I. Christenson, *Acta Pharm. Suec.*, **6**, 287 (1969).

(6) R. D. O'Brien, B. D. Hilton, and L. Gilmour, *Mol. Pharm.*, **2**, 593 (1966).

(7) R. D. O'Brien, *ibid.*, **4**, 121 (1968).

(8) F. F. Foldes and J. C. Smith, *Ann. N.Y. Acad. Sci.*, **135**, 287 (1966).

(9) F. P. W. Winteringham and K. S. Fowler, *Biochem. J.*, **101**, 127 (1966).

(10) O. E. Schultz and R. Kratzer, *Arch. Pharm.*, **295**, 444 (1962).

(11) S. Udenfriend, D. E. Duggan, B. M. Vasta, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **120**, 26 (1957).

(12) J. D. Winefordner, in "Fluorescence and Phosphorescence Analysis; Principles and Applications," D. M. Hercules, Ed., Wiley, New York, N.Y., 1966, p. 169.

(13) A. R. Rogers and G. Smith, *J. Chromatogr.*, **87**, 125 (1973).

(14) W. A. Groff, A. Kaminskis, and R. I. Ellin, *Clin. Toxicol.*, **9**, 353 (1976).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 1, 1976, from the *Biomedical Laboratory, Edgewood Arsenal, Aberdeen Proving Ground, MD 21010.*

Accepted for publication May 18, 1976.

The views of the authors do not purport to reflect the position of the Department of Defense. The use of trade names does not constitute an official endorsement or approval of such commercial hardware or software. This report may not be cited for advertisement purposes.

[x] To whom inquiries should be directed.

Influence of Zinc–Ligand Mixtures on Serum Zinc Levels in Rats

EUGENE GIROUX [x] and N. J. PRAKASH

Abstract □ The influence of various salts, chelates, and other complexes of zinc given by gavage on serum zinc levels in rats was studied. Serum zinc concentrations were determined over 6 hr after administration of zinc sulfate at doses of 5, 10, 20, 50, and 100 mg of zinc/kg. Serum zinc levels following doses of zinc salts and complexes were compared with those after zinc sulfate. Phytic acid, aminopolycarboxylic acids (including edetate disodium), and penicillamine significantly suppressed increases in serum zinc concentration. Some natural amino acid–zinc sulfate mixtures (those with lysine, cysteine, glycine, and histidine) produced greater levels than the equivalent dose of zinc sulfate alone. Several thiocarboxylic acids, such as mercaptoacetic acid and thiosalicylic acid, also increased serum zinc concentrations. These observations form a basis for attempted modification of zinc absorption in other species.

Keyphrases □ Zinc—serum levels, effect of various salts, chelates, and other complexes, rats □ Salts, zinc—effect on serum zinc levels, rats □ Chelates, various—with zinc, effect on serum zinc levels, rats □ Complexes, various—with zinc, effect on serum zinc levels, rats □ Metals—zinc, various salts, chelates, and other complexes, effect on serum zinc levels, rats

Treatment of humans with zinc usually involves administration of zinc sulfate heptahydrate, taken with meals to avoid gastric irritation. However, Schelling *et al.* (1) reported that when it is given simultaneously with meals, the apparent absorption of zinc may be nil. Poor absorption may partly account for equivocal clinical results of zinc therapy (2–7).

GI absorption of zinc in several species has been studied

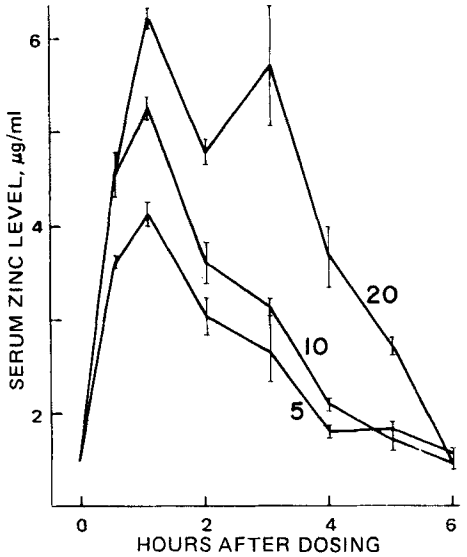

Figure 1—*Serum zinc levels following gavage of 5, 10, and 20 mg of zinc/kg, given as zinc sulfate heptahydrate dissolved in water. Points indicate mean values; vertical bars denote ± SEM. At least five rats were used for each point except for the 1- and 4-hr points at 5 mg of zinc/kg, where three and four rats were used, respectively.*

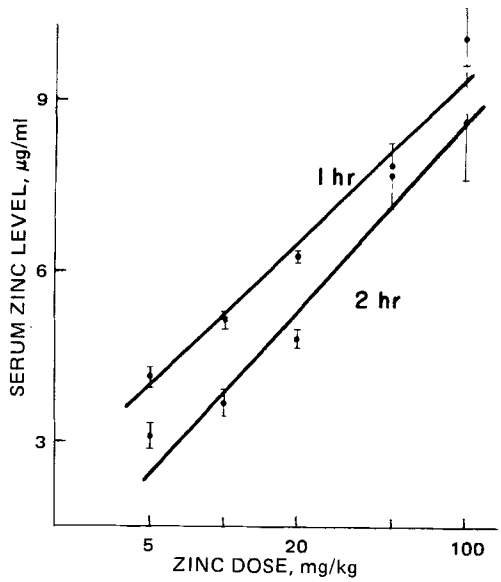

Figure 2—*Serum zinc levels 1 and 2 hr after gavaging of zinc sulfate heptahydrate dissolved in water. (See Fig. 1 for further explanation.)*

in vivo and *in vitro*. Various experimental designs have been employed including conventional balance studies, radioisotopic tracer studies, and studies of the bioavailability of zinc from foodstuffs as monitored by correction of zinc deficiency symptoms. Zinc absorption was discussed in several reviews (8–12). In one study, edetate and ascorbic acid depressed gut mucosal uptake of zinc but enhanced transmural transport during *in vitro* perfusion experiments on rats (13). Zinc–edetate complexes and zinc–histidine complexes were absorbed intact from the gut and appeared in the plasma of chickens further complexed with plasma proteins (14). Zinc–aspirin complexes were broken down and did not appear intact in serum (14).

Apparently, some compounds potentiate zinc absorption when given with an oral dose of zinc sulfate while other substances suppress it. Accordingly, serum zinc levels were measured in rats after gavage of mixtures of zinc sulfate with various complexing agents. Compounds with different zinc coordinating sites were studied including thiol, amino, and carboxylic acid groups. Phytic acid and various amino acids were chosen because of their relevance to trace metal metabolism.

EXPERIMENTAL

Materials[1]—Reagents and chemicals, including bis[*N*,*N*-di(carboxymethyl)aminoethoxy]ethane (I), the ammonium salt of pyrrolidine-*N*-carboxydithioic acid (II), and 3-mercaptopropionylglycine (III), were obtained commercially, except for *N*-acetylhydroxyproline, which was prepared in this laboratory.

Animals and *In Vivo* Procedure—Male Sprague–Dawley rats[2], 140–240 g, were maintained in open mesh, stainless steel cages for at least 1 week in the laboratory environment (12-hr dark cycle beginning at 7 pm and controlled temperature and humidity). They were fed laboratory ration[3] and tap water *ad libitum* until 20–24 hr before an experiment. From that time and throughout the experiment, animals were fasted and allowed access only to demineralized water.

Each experiment began at 9–10 am. Various zinc mixtures were introduced by gavage. After 0.5–6 hr, animals were lightly anesthetized with ether and blood was taken from the heart with plastic syringes and stainless steel needles. The blood was allowed to clot in acid-washed glass tubes, and serum was obtained by centrifugation. A second centrifugation removed from the serum traces of erythrocytes carried over from the initial centrifugation.

Aliquots of serum were treated with trichloroacetic acid to a final concentration of 5% (15), and protein-free supernates were analyzed for zinc by atomic absorption spectrophotometry[4]. Zinc contamination was reduced through use of plastic zinc-free labware, disposable plastic test tubes, acid-washed pipets, and demineralized, double-distilled water for preparation of all reagents. Statistical comparison of means was by the Student two-tailed *t*-test with the assumption that variances of the populations were not necessarily identical (16).

Preparation of Mixtures—With few exceptions, test mixtures were prepared by combining aqueous zinc sulfate with an aqueous solution (or suspension) of the other component. A few mixtures were adjusted to pH 5–6, but the pH of the test mixtures generally was not controlled. The concentration of zinc in the final mixture was always chosen so that the volume administered was 5 ml/kg. If the mixture contained insoluble components, it was homogenized[5] immediately prior to gavage.

Butanol Solubility Measurements—Solubility of zinc in an organic phase as modified by the presence of ligand was determined by partitioning zinc-65 between 1-butanol and an aqueous solution [0.1 *M* NaCl–0.1 *M* 2-(*N*-morpholino)ethanesulfonic acid] buffered at pH 6.5. Each mixture contained 2 ml of aqueous buffer, 2.5 ml of 1-butanol, 5 μmoles of complexing agent, and 2 μmoles of ^{65}Zn-labeled zinc sulfate. Butanol and aqueous phases were repeatedly mixed and then separated by centrifugation. An aliquot of the butanol phase was counted in a γ-spectrometer[6].

[1] Anthranilic acid and 1-butanol from J. T. Baker, Deventer, Holland; 8-hydroxyquinoline, 8-hydroxyquinoline-5-sulfonic acid, 2-mercapto-1-methylimidazole, 3-mercaptopropionic acid, III, penicillamine, and thiosalicylic acid from Fluka, Buchs, Switzerland; 2-(*N*-morpholino)ethanesulfonic acid from Hopkin and Williams, Essex, England; acetylacetone from K & K Laboratories, Plainsview, N.Y.; asparagine, citric acid, cysteine, galacturonic acid, glycine, histidine, β-hydroxybutyric acid, imidazole, lysine, oxalic acid, trichloroacetic acid, and zinc sulfate heptahydrate from E. Merck, Darmstadt, Germany; ^{65}Zn-labeled zinc chloride from NEN Chemicals, GmbH, Dreieichenhain, Germany; malonic acid and salicylic acid from Prolabo, Paris, France; edetate disodium and II from Karl Roth, Karlsruhe, Germany; *N*-acetylcysteine, β-alanine, and I from Serva, Heidelberg, Germany; 2,4-diaminobutyric acid, dithioerythritol, ethylenediaminediacetic acid, nitrilotriacetic acid, sodium phytate, and 1,2-transcyclohexyldiaminetetraacetic acid from Sigma Chemical Co., St. Louis, Mo.; all others from Aldrich-Europe, Beerse, Belgium.

[2] Charles River France, St.-Aubin-des-Elbeuf.

[3] Diet A 04 (containing 85 mg of zinc/kg), U.A.R., Villemoisson-sur-Orge, France.

[4] Perkin-Elmer model 403 atomic absorption spectrophotometer.

[5] Potter–Elvehjem apparatus.

[6] Auto-Gamma scintillation spectrometer, Packard Instrument Co.

Table I—Serum Zinc Levels after Gavage of Zinc Sulfate–Ligand Mixtures at a Dose of 10 mg of Zinc/kg

Ligand	Zinc–Ligand Molar Ratio	Serum Zinc, μg/ml (Mean ± *SEM*)[a] 1 hr	4 hr
Sodium phytate	1:1	1.50 ± 0.02 (6)[b]	1.69 ± 0.03 (5)[b]
I	1:1	1.94 ± 0.12 (6)[b]	1.84 ± 0.27 (4)
8-Thioquinoline	1:2	2.06 ± 0.10 (6)[b]	—[c]
Penicillamine	1:2	2.06 ± 0.15 (5)[b]	1.54 ± 0.13 (5)[b]
Oxalic acid	1:1	2.39 ± 0.19 (5)[b]	1.76 ± 0.08 (4)
Edetate disodium	1:1	2.60 ± 0.13 (5)[b]	1.75 ± 0.17 (5)
1,3-Dimercapto-2-propanol	1:1	2.65 ± 0.19 (6)[b]	3.85 ± 0.34 (5)[d]
Dithioerythritol	1:1	2.77 ± 0.08 (6)[b]	3.02 ± 0.48 (5)[d]
2,3-Dimercapto-1-propanol	1:1	3.43 ± 0.24 (6)[b]	2.46 ± 0.13 (5)
8-Hydroxyquinoline	1:2	3.60 ± 0.20 (6)[b]	2.48 ± 0.09 (4)
2,3-Diaminopyridine	1:2	3.70 ± 0.20 (6)[b]	4.45 ± 0.22 (10)[d]
II	1:2	3.79 ± 0.26 (6)[b]	4.51 ± 0.20 (5)[d]
III	1:1	3.99 ± 0.22 (6)[b]	2.10 ± 0.18 (5)
2-Pyridylacetic acid	1:2	4.22 ± 0.22 (5)[b]	2.79 ± 0.22 (5)[d]
Anthranilic acid	1:1	4.31 ± 0.13 (6)[b]	2.39 ± 0.19 (5)
N-Acetylcysteine	1:2	4.72 ± 0.33 (6)	2.09 ± 0.15 (5)
3-Mercaptopropionic acid	1:1	4.96 ± 0.41 (6)	3.08 ± 0.18 (5)[d]
Salicylic acid	1:1	5.02 ± 0.10 (6)	2.33 ± 0.16 (5)
2-Mercapto-1-methylimidazole	1:2	5.03 ± 0.37 (6)	3.20 ± 0.23 (5)[d]
N-Acetylhydroxyproline	1:1	5.07 ± 0.34 (5)	2.14 ± 0.22 (5)
Acetylacetone	1:2	5.15 ± 0.22 (5)	4.06 ± 0.32 (5)[d]
8-Hydroxyquinoline-5-sulfonic acid	1:2	5.27 ± 0.20 (6)	2.81 ± 0.28 (5)[d]
Zinc sulfate heptahydrate (control)		5.29 ± 0.12 (15)	2.12 ± 0.06 (16)
2,4-Diaminobutyric acid	1:1	5.41 ± 0.23 (6)	2.29 ± 0.10 (5)
Asparagine	1:2	5.42 ± 0.16 (6)	2.77 ± 0.24 (5)
β-Alanine	1:2	5.46 ± 0.25 (6)	2.06 ± 0.15 (5)
Histidine methyl ester	1:2	5.56 ± 0.24 (12)	2.24 ± 0.16 (10)
3,4-Diaminobenzoic acid	1:2	5.59 ± 0.33 (5)	3.37 ± 0.27 (5)[d]
1,3-Diamino-2-propanol	1:2	5.78 ± 0.22 (6)	2.28 ± 0.25 (5)
Imidazole	1:2	5.87 ± 0.17 (6)	2.46 ± 0.12 (5)
Lysine	1:2	5.91 ± 0.13 (6)[d]	2.38 ± 0.07 (5)
Cysteine	1:2	5.97 ± 0.15 (12)[d]	—[c]
Citric acid	1:1	6.02 ± 0.37 (6)	—[c]
Histidine	1:2	6.23 ± 0.58 (6)	—[c]
Glycine	1:2	6.61 ± 0.42 (6)[d]	—[c]
Malonic acid	1:1	6.66 ± 0.24 (11)[d]	2.63 ± 0.11 (14)[d]
Mercaptoacetic acid	1:2	6.68 ± 0.46 (5)[d]	3.71 ± 0.54 (4)[d]
2-Mercapto-3-pyridinol	1:2	6.76 ± 0.17 (5)[d]	3.19 ± 0.24 (5)[d]
β-Hydroxybutyric acid	1:2	6.77 ± 0.46 (10)[d]	2.95 ± 0.24 (5)
Mercaptosuccinic acid	1:1	6.83 ± 0.28 (6)[d]	2.31 ± 0.14 (5)
α-D-Galacturonic acid	1:2	7.22 ± 0.42 (6)[d]	2.18 ± 0.18 (5)
Thiosalicylic acid	1:1	9.93 ± 0.36 (22)[d]	4.28 ± 0.28 (15)[d]

[a] The numbers in parentheses are the number of animals. [b] Significantly below control value, $p < 0.005$. [c] Data obtained 3 hr following dosing were not significantly different from control values. [d] Significantly above control value, $p < 0.005$.

RESULTS

Serum Zinc Levels following Zinc Sulfate—The serum zinc concentration was determined 1, 2, 3, 4, and 6 hr after gavage of rats with demineralized water. The mean values at these points were not significantly different from one another; the overall mean (28 rats) was 1.50 ± 0.03 (*SEM*) μg of zinc/ml of serum. Serum zinc levels were determined at 0.5, 1, 2, 3, 4, 5, and 6 hr following doses of 5, 10, 20, 50, and 100 mg of zinc/kg, given as aqueous solutions of zinc sulfate heptahydrate.

Serum concentration–time curves after 5, 10, and 20 mg of zinc/kg are shown in Fig. 1. With these doses, maximum serum zinc concentrations were observed 1 hr after gavage, and the level returned to the baseline concentration within 6 hr. Semilogarithmic curves relating all doses administered to the resulting serum concentrations were approximately linear (Fig. 2). The data indicated that measurable increases in serum zinc concentrations would not be observed at doses less than 1–2 mg of zinc/kg. Peak serum levels of 20 μg of zinc/ml (approximately 15 times greater than normal) did not produce any lethality over 8 hr.

Serum Zinc Levels following Zinc–Ligand Mixtures—Serum zinc concentrations were measured 1 and 4 hr following gavage (Table I). Doses of test mixtures contained 10 mg of zinc/kg, and the molar ratio of zinc to ligand was either 1:1 or 1:2. Serum zinc levels following gavage of zinc–ligand mixtures ranged from little or no increase above serum zinc levels in untreated animals to highly significant increases beyond the increase caused by zinc sulfate alone. Aminopolycarboxylic acids of the edetate type suppressed increases in serum zinc concentrations. Other aminopolycarboxylic acids tested in addition to those listed in Table I were nitrilotriacetic acid, ethylenediaminediacetic acid, and 1,2-*trans*-cyclohexyldiaminetetraacetic acid. These chelators also significantly ($p < 0.005$) diminished the increase in the serum zinc level.

The dithiol compounds 1,3-dimercapto-2-propanol, 2,3-dimercapto-1-propanol, and dithioerythritol significantly inhibited the elevation of serum zinc levels at 1 hr, but the levels were greater than the control value after 4 hr. After gavage of several compounds listed in Table I, serum levels of zinc were greater than those following equivalent doses of zinc sulfate. Notable among these latter compounds were some natural amino acids (lysine, glycine, cysteine, and histidine) and compounds containing thiol and carboxylic acid groups such that five- and six-membered chelate complexes with zinc could be formed.

Time–concentration curves following gavage of mixtures of zinc sulfate with penicillamine and with thiosalicylic acid are shown in Fig. 3A. Maximum serum zinc levels were noted 1 hr after dosing, with return to baseline concentration within 6 hr. When given in a mixture with zinc sulfate, D,L-penicillamine significantly ($p < 0.005$) suppressed the increase in the serum zinc level while thiosalicylic acid significantly ($p < 0.005$) enhanced the increase. The levels following gavage of ligands in the absence of zinc sulfate also were determined. Penicillamine reduced and thiosalicylic acid increased the serum zinc levels (Fig. 3B).

Time–serum zinc concentration curves for sodium phytate and β-hydroxybutyric acid given with and without zinc sulfate were obtained similarly (Fig. 4). Areas between the curves (Figs. 3 and 4) in the presence and absence of zinc sulfate were calculated: zinc sulfate (control), 9.2; thiosalicylic acid, 21.1; β-hydroxybutyric acid, 11.5; penicillamine, 3.8; and sodium phytate, 0.1 μg hr/ml. These results are consistent with the hypothesis that thiosalicylic acid and β-hydroxybutyric acid increase zinc absorption while penicillamine and sodium phytate suppress zinc absorption from zinc sulfate heptahydrate.

Butanol Solubility of Zinc–Ligand Mixtures—Partitioning of zinc-65 between aqueous buffer and butanol was compared for poorly absorbed and well-absorbed zinc–ligand mixtures. Ligands that sup-

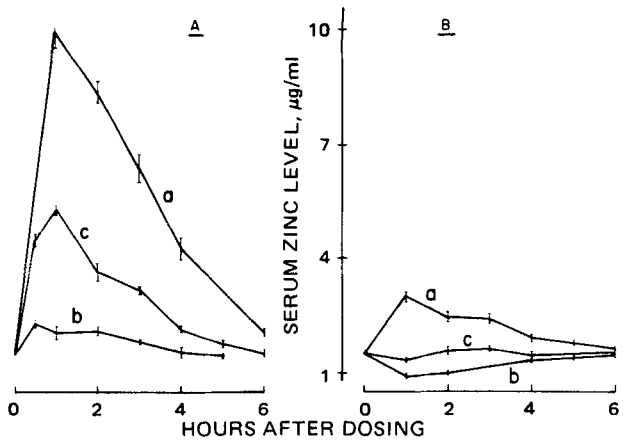

Figure 3—(*A*) *Serum zinc levels following gavage of zinc sulfate* (*c*) (*10 mg of zinc/kg*) *given alone or mixed with* D,L-*penicillamine* (*b*) (*1:2 molar ratio*) *or thiosalicylic acid* (*a*) (*1:1 molar ratio*). (*B*) *Serum zinc levels following gavage of thiosalicylic acid* (*a*) *or penicillamine* (*b*) *at the same concentrations as in A or of distilled water* (*c*). *Points* (*at least five rats per point*) *indicate mean values; vertical bars denote* ± SEM.

pressed zinc absorption also tended to suppress zinc solubility in the butanol phase (Table II), as did some ligands that augmented zinc absorption. In the absence of ligand, 0.9% of the ^{65}Zn-radioactivity was recovered in the butanol phase.

DISCUSSION

Ligands markedly influenced the serum zinc levels following their administration by gavage together with zinc sulfate. While these ligands might influence distribution or elimination of zinc after their absorption, it seems more likely that their major effect is on zinc absorption from the GI tract, particularly when the time course of serum zinc concentrations after dosing with ligands is similar to that seen after zinc sulfate. Thus, the form in which zinc is presented to the GI tract apparently influences zinc absorption. Phytic acid, considered responsible for dietary zinc deficiencies reported in Near Eastern adolescents (17), completely prevented increases in the serum zinc levels when coadministered with zinc sulfate. Most α-amino acids enhanced the increases. Penicillamine markedly suppressed such increases, but the structurally similar cysteine enhanced them. These two compounds form equally stable zinc complexes (18). For some mixtures of zinc sulfate and ligand, serum zinc levels at 4 hr were greater than at 1 hr after gavage; thus, the temporal pattern of zinc levels after these mixtures did not resemble that after zinc sulfate.

Constants for zinc complex formation are available (18) for several tested compounds. There was no obvious relationship between the effect on serum zinc levels and the complex formation constant, although some of the strongest complexes were among the most poorly absorbed mixtures. Furthermore, the solubility of the complexes in the test mixture did not seem to be the decisive factor. The water-soluble edetate–zinc mixture suppressed apparent zinc absorption, as did the insoluble 2,3-dimercapto-1-propanol–zinc mixture. The insoluble thiosalicylic acid–zinc mixture appeared significantly effective in enhancing zinc absorption. Lipid solubility of zinc species presented to GI absorption sites may play a role in absorption, but the data on the effect of ligands on butanol solubility of zinc are inconclusive in support of this hypothesis.

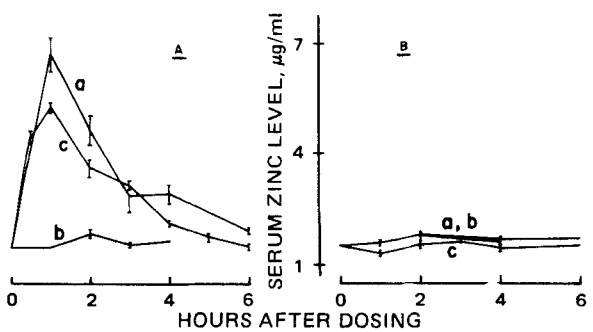

Figure 4—(*A*) *Serum zinc levels following gavage of zinc sulfate* (*c*) (*10 mg of zinc/kg*) *given alone or mixed with sodium phytate* (*b*) (*1:1 molar ratio*) *or β-hydroxybutyric acid* (*a*) (*1:2 molar ratio*). (*B*) *Serum zinc levels following gavage of β-hydroxybutyric acid* (*a*) *or sodium phytate* (*b*) *at the same concentrations as in A or of distilled water* (*c*). *Points* (*at least four rats per point*) *indicate mean values; vertical bars denote* ± SEM.

Table II—Stability and Butanol Solubility of Zinc Complexes

Ligand	Apparent Log K_1 pH 6.5[a]	Butanol-Soluble ^{65}Zn[b], %
Sodium phytate	—	<0.1
I	9.2	<0.1
8-Thioquinoline	8.3	55
Penicillamine	4.2	0.9
Oxalic acid	4.9	0.3
Edetate disodium	12.5	<0.1
1,3-Dimercapto-2-propanol	—	<0.1
Dithioerythritol	—	0.1
2,3-Dimercapto-1-propanol	7.3	0.5
8-Hydroxyquinoline	5.1	37
Cysteine	3.8	0.5
Histidine	3.8	1.3
Glycine	2.0	0.9
Malonic acid	2.8	0.9
Mercaptoacetic acid	4.2	1.4
2-Mercapto-3-pyridinol	—	85
β-Hydroxybutyric acid	1.1	1.0
Mercaptosuccinic acid	4.6	<0.1
α-D-Galacturonic acid	1.7[c]	0.8
Thiosalicylic acid	6.1	34

[a] Data were taken from Ref. 18; values at pH 6.5 were calculated as described in Ref. 22. [b] Distribution of isotope in presence of ligand between aqueous and 1-butanol phases as described in the text; each value is the average of two determinations. Butanol-soluble isotope in absence of ligand, 0.9%. [c] Ref. 19.

Makar *et al.* (19) suggested that oxalic acid, galacturonic acid, and β-hydroxybutyric acid increase zinc absorption because of the preponderance of uncharged complexes these compounds form with zinc ions at neutral pH. The present results support their suggestion, with the exception that oxalic acid suppressed zinc absorption. Quite possibly, all factors previously considered contribute to the overall effect of the ligands on zinc absorption.

These observations apply only to the rat. However, in other species, oral intake of certain zinc–ligand mixtures may also increase serum levels of zinc more than oral zinc sulfate treatment. Alternatively, a smaller amount of zinc–ligand mixture may provide the equivalent effect of a greater amount of zinc sulfate, thereby reducing GI side effects of oral zinc sulfate therapy. Ligand effects may also partly account for diminished apparent zinc absorption when zinc sulfate is administered with meals (1, 20, 21).

REFERENCES

(1) J. L. Schelling, S. Muller-Hess, and F. Thonney, *Lancet*, **2**, 968 (1973).

(2) K. Haeger, E. Lanner, and P. O. Magnusson, *VASA, J. Vasc. Dis.*, **1**, 62 (1972).

(3) S. L. Husain, *Lancet*, **1**, 1069 (1969).

(4) G. R. Sergeant, R. E. Galloway, and M. C. Gueri, *ibid.*, **2**, 891 (1970).

(5) T. Hallböök and E. Lanner, *ibid.*, **2**, 891 (1970).

(6) B. M. Myers and G. Cherry, *Am. J. Surg.*, **120**, 77 (1970).

(7) M. W. Greaves and F. A. Ive, *Br. J. Dermatol.*, **87**, 632 (1972).

(8) B. L. O'Dell, *Am. J. Clin. Nutr.*, **22**, 1315 (1969).

(9) W. G. Hoekstra, in "Trace Element Metabolism in Animals," C. F. Mills, Ed., E. and S. Livingstone, Edinburgh, Scotland, 1970, pp. 347–353.

(10) E. J. Underwood, "Trace Elements in Human and Animal Nutrition," 3rd ed., Academic, New York, N.Y., 1971, pp. 208–252.

(11) W. M. Becker and W. G. Hoekstra, in "Intestinal Absorption of Metal Ions, Trace Elements and Radionuclides," S. C. Skoryna and D. W. Edwards, Eds., Pergamon, Oxford, England, 1971, pp. 229–256.

(12) B. L. O'Dell, *Ann. N.Y. Acad. Sci.*, **199**, 70 (1972).

(13) B. M. Sahagian, I. Harding-Barlow, and H. M. Perry, Jr., *J. Nutr.*, **93**, 291 (1967).

(14) F. A. Suso and H. M. Edwards, Jr., *Nature*, **236**, 230 (1972).
(15) I. J. T. Davies, M. Musa, and T. L. Dormandy, *J. Clin. Pathol.*, **21**, 359 (1968).
(16) R. D. Remington and M. A. Schork, "Statistics with Applications to the Biological and Health Sciences," Prentice-Hall, Englewood Cliffs, N.J., 1970, pp. 212, 213.
(17) J. G. Reinhold, K. Nasr, A. Lahimgarzadeh, and H. Hedayati, *Lancet*, **1**, 283 (1973).
(18) L. G. Sillén and A. E. Martell, "Stability Constants of Metal-Ion Complexes," The Chemical Society Special Publication No. 17, London, England, 1964. L. G. Sillén and A. E. Martell, "Stability Constants of Metal-Ion Complexes," Supplement No. 1, The Chemical Society Special Publication No. 25, London, England, 1971.
(19) G. K. R. Makar, M. L. D. Touche, and D. R. Williams, *J. Chem. Soc. Dalton Trans.*, **1976**, 1016.
(20) A. Pécoud, P. Donzel, and J. L. Schelling, *Clin. Pharmacol. Ther.*, **17**, 469 (1975).
(21) F. J. Oelshlegel, Jr., and G. J. Brewer, *Clin. Res.*, **23**, 2221 (1975).
(22) W. J. O'Sullivan, in "Data for Biochemical Research," 2nd ed., R. M. C. Dawson, D. C. Elliott, W. H. Elliott, and K. M. Jones, Eds., Clarendon, Oxford, England, 1969, pp. 423–434.

ACKNOWLEDGMENTS AND ADDRESSES

Received March 10, 1976, from *Centre de Recherche Merrell International, 16, rue d'Ankara, F 67000 Strasbourg, France.*
Accepted for publication May 19, 1976.
[x] To whom inquiries should be directed.

Pharmacokinetics of Δ^9-Tetrahydrocannabinol in Dogs

EDWARD R. GARRETT [x] and C. ANTHONY HUNT [x]

Abstract □ The pharmacokinetics of intravenously administered ^{14}C-Δ^9-tetrahydrocannabinol and derived radiolabeled metabolites were studied in three dogs at two doses each at 0.1 or 0.5 and 2.0 mg/kg. Two dogs were biliary cannulated; total bile was collected in one and sampled in the other. The time course for the fraction of the dose per milliliter of plasma was best fit by a sum of five exponentials, and there was no dose dependency. No drug was excreted unchanged. The mean apparent volume of distribution of the central compartment referenced to total drug concentration in the plasma was 1.31 ± 0.07 liters, approximately the plasma volume, due to the high protein binding of 97%. The mean metabolic clearance of drug in the plasma was 124 ± 3.8 ml/min, half of the hepatic plasma flow, but was 4131 ± 690 ml/min referenced to unbound drug concentration in the plasma, 16.5 times the hepatic plasma flow, indicating that net metabolism of both bound and unbound drug occurs. Apparent parallel production of several metabolites occurred, but the pharmacokinetics of their appearance were undoubtedly due to their sequential production during liver passage. The apparent half-life of the metabolic process was 6.9 ± 0.3 min. The terminal half-life of Δ^9-tetrahydrocannabinol in the pseudo-steady state after equilibration in an apparent overall volume of distribution of 2170 ± 555 liters referenced to total plasma concentration was 8.2 ± 0.23 days, based on the consistency of all pharmacokinetic data. The best estimate of the terminal half-life, based only on the 7000 min that plasma levels could be monitored with the existing analytical sensitivity, was 1.24 days. However, this value was inconsistent with the metabolite production and excretion of 40–45% of dose in feces, 14–16.5% in urine, and 55% in bile within 5 days when 24% of the dose was unmetabolized and in the tissue at that time. These data were consistent with an enterohepatic recirculation of 10–15% of the metabolites. Intravenously administered radiolabeled metabolites were totally and rapidly eliminated in both bile and urine: 88% of the dose in 300 min with an apparent overall volume of distribution of 6 liters. These facts supported the proposition that the return of Δ^9-tetrahydrocannabinol from tissue was the rate-determining process of drug elimination after initial fast distribution and metabolism and was inconsistent with the capability of enzyme induction to change the terminal half-life.

Keyphrases □ Δ^9-Tetrahydrocannabinol—intravenous, radiochemical study of pharmacokinetics, dogs □ Pharmacokinetics—intravenous, Δ^9-tetrahydrocannabinol, radiochemical study, dogs □ Radiochemistry—study of pharmacokinetics of intravenous Δ^9-tetrahydrocannabinol, dogs

(−)-Δ^9-Tetrahydrocannabinol is the major active component of marijuana. An essential prerequisite to understand its pharmacological action, presumably related to the plasma levels of the drug and its metabolites (1, 2), is the quantification of the time course of the drug and its metabolites in biological tissues to relate to the psychoactive effects.

Agurell *et al.* (3, 4) demonstrated that when tritium-labeled Δ^9-tetrahydrocannabinol was intravenously administered to the mouse and rabbit, the radioactivity was slowly excreted as metabolites in the feces and urine with no unchanged drug observed in the urine. The relative amounts excreted biliary and renally varied with the species (3–5), and enterohepatic recirculation of metabolites was indicated (5, 6). The major studies to date with pharmacokinetic significance are those of Agurell *et al.* (4, 5) in rabbits and humans and Lemberger *et al.* (5, 7, 8) in humans. The general pattern appears to be a rapid initial fall of Δ^9-tetrahydrocannabinol concentration in plasma with an apparent half-life of 12 min in rabbits (4) and 30 min in humans (8), with a slower decline to a terminal apparent half-life of 50–60 hr in humans (8). A similar pattern was observed in a preliminary experiment with dogs (9). Metabolite levels in human plasma increased rapidly to two to three times that of the drug (8). The slower terminal phase decline of the log metabolite level with time paralleled the similarly plotted decline of plasma Δ^9-tetrahydrocannabinol.

It has been proposed (7, 10) that these facts can be explained by concomitant rapid hepatic metabolism and distribution to binding sites and other tissues such as fat (11) with a subsequent slow release of sequestered Δ^9-tetrahydrocannabinol from these stores. The suggestion that induced metabolism significantly lessens the terminal half-life of Δ^9-tetrahydrocannabinol in chronic users over that of naive individuals (2, 12) is inconsistent with this premise. The release rate from the "deep" tissues should be rate determining, not the metabolic rate. Changes in the latter would merely affect the relative plasma Δ^9-tetrahydrocannabinol levels, not the terminal half-life.

The purposes of this paper are to present the results of studies designed to elucidate the rate-determining pro-