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Kinetics of the Intestinal Uptake of Zinc Acexamate in Normal and Zinc-depleted Rats

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Abstract—The uptake of zinc as acexamic acid salt in the small intestine of the anaesthetized rat was shown to be a two-phase process in normal animals. The first phase is rapid mucosal binding which satisfies the Freundlich isotherm equation and which involves about 30 per cent of the initially perfused zinc. The second phase was characterized as an apparent absorption step which obeys Michaelis-Menten and first-order combined kinetics, with the following parameters: $V_m = 6{\cdot}51$ mg h^{-1}; $K_m = 2{\cdot}96$ mg; $k_a = 0{\cdot}306$ h^{-1}. In largely non-saturated conditions, an apparent global rate constant of about 2·50 h^{-1} was calculated. No significant interference due to endogenous zinc excretion into the small intestine was observed during the absorption period. In zinc-deficient animals, the two phases were not so well characterized. Binding was non-linear and apparent absorption efficiency was much greater at high zinc concentrations, so no evidence of saturable kinetics was found, thus confirming the hypothesis of a homeostatic zinc regulation mechanism.

In the two last decades, zinc metabolism in animals and man has been extensively studied (Cousins 1986). However, zinc transport in the small intestine, from mucosa to serosa (i.e. zinc absorption) and from serosa to mucosa (i.e. endogenous zinc excretion into the small intestine, a natural zinc emunctory) are still poorly understood phenomena. These processes are, indeed, of outstanding importance in the nutritional field. Recently, interest has focused on the pharmacological field with pharmacokinetic studies on zinc bioavailability and dosage schedules in the treatment and prevention of peptic ulcers, whether of idiopathic or iatrogenic origin. The acexamic acid salt has been extensively used for this purpose (Frommer 1975; Alcalá et al 1985; Varas 1986).

The amounts, rates, mechanisms and kinetics of intestinal zinc absorption have been studied in animals, using in-vitro procedures on everted intestinal segments (Kowarski et al 1974; Davies 1980; Seal & Heaton 1983), in-situ methods with isolated, vascularly perfused intestinal fractions (Antonson et al 1979; Smith & Cousins 1980; Oestreicher & Cousins 1982; Steel & Cousins 1985; Hoadley et al 1987) and in-vivo intubation techniques (Faraji & Swendseid 1983). The clearly distinct characteristics of zinc management by normal and zinc-depleted animals has led to the almost generalized agreement that zinc incorporation into the body is under homeostatic control. Accumulated evidence on dietary zinc suggests that zinc intestinal uptake in the rat is a carrier-mediated process (Davies 1980; Smith & Cousins 1980) probably combined with a non-saturable diffusion mechanism (Steel & Cousins 1985; Seal & Heaton 1987; Hoadley et al 1987). The excretion of endogenous zinc into the small intestine has been reported to be highly significant and may interfere with absorption rate measurements (Evans et al 1979; Weigand & Kirchgessner 1980).

As far as zinc administration in therapeutics is concerned the main research objective is to ascertain absorption kinetics of zinc maintenance doses to enable the development of simple and reliable models to study the physiological processes involved in zinc absorption and excretion. The rat in-situ gut preparation as adapted here, may be a good approach as it would retain the intestinal ability to exercise a homeostatic control over the zinc absorption/excretion processes. The technique has been tested in our laboratory to characterize carrier-mediated absorption kinetics with quite acceptable results (Merino et al 1989a, b; Sánchez-Picó et al 1989) and has proved useful in providing guidance on human bioavailability trials (Garrigues et al 1990).

In this paper the technique is applied to the determination of uptake in the whole small intestine in normal and zinc-deficient rats.

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Materials and Methods

Zinc salt

The experiments were carried out with zinc acexamate (zinc ε-acetamido caproate, $Zn(CH_3CONH(CH_2)_5COO)_2$).

Animals

Tests were carried out on two groups of male Wistar albino rats: (a) normal animals, 330–360 g, fed a commercially standardized diet containing 85 mg kg^{-1} zinc (Panlab A-04), and (b) zinc-depleted animals, 290–330 g, receiving a specially prepared zinc-deficient diet (Panlab 2·8 mg kg^{-1} Zn) for 12 days before surgery. Zinc deficiency was verified by the classical symptoms of hair loss and weight decrease during the deprivation period (Sandstead et al 1976; Faraji & Swendseid 1983). Representative growth curves for each group are shown in Fig. 1.

Treatment of material and reagents

To prevent or minimize zinc contamination, glassware was avoided wherever possible (Smith et al 1979; Alcock 1981) and only glass volumetric flasks and pipettes were used;

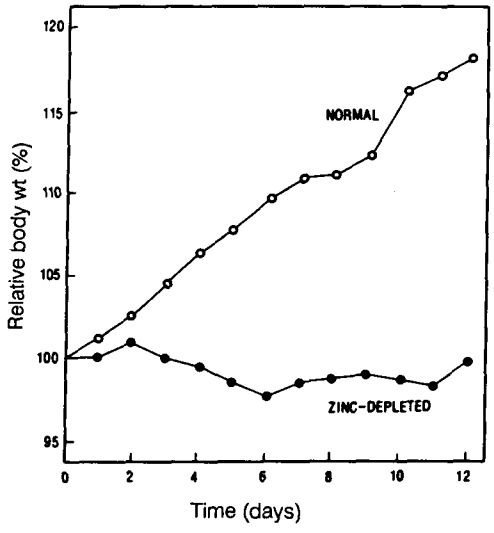

FIG. 1. Representative growth curves found for two groups of animals over 12 days (means of six animals per group). The deprivation period for zinc-depleted animals started at zero time.

otherwise, polypropylene material was employed for laboratory operations.

All labware was first washed with soap and tap water, rinsed, maintained in a 20% nitric acid solution for 24 h, then rinsed with deionized water and heated to 40°C (120°C for glassware) before use.

Reagents were analytical grade, free or practically free of zinc.

Test solutions

Perfusion solutions were prepared from a concentrated isotonic zinc solution (1500 μg mL^{-1}), buffered with 20 mM HEPES (hydroxyethyl-piperazine-*N'*-2-ethanedisulphonic acid). The composition of the solution was: zinc acexamate 940·2 mg, HEPES 476·8 mg, sodium chloride 592·3 mg, deionized water, up to 100·0 mL.

After dilution with 0·9% NaCl (saline), also buffered with HEPES, solutions containing 1000, 500, 50, 25 and 5 μg mL^{-1} of zinc were prepared as perfusion fluids.

Tonicity of solutions was assessed immediately after preparation with the aid of a Halb-Micro Haver osmometer, and adjusted when necessary. Immediately before each intestinal perfusion, the pH of the solution was adjusted to 6·2 with 2 M solution of Tris (tris-(hydroxymethyl) aminomethane) in isotonic saline. The use of such solutions prevents any substantial variation in pH, which could lead to zinc precipitation in the intestinal perfusion fluids during the absorption experiments.

Experimental absorption technique

The anaesthetized rat gut preparation recommended by Doluisio et al (1969), as modified previously (Martin-Villodre et al 1986) was used with a total perfusion volume of 10 mL. To prevent recycling, the biliary duct was ligated.

In normal animals, solutions containing 1500, 1000, 500, 50 and 5 μg mL^{-1} of zinc were perfused (5 animals per set); in zinc-depleted animals only solutions containing 1000, 500 and 25 μg mL^{-1} of zinc were used for perfusions because of the limited availablity of the zinc-deficient diet (5 animals per set). The series were handled independently, but simultaneously.

Some reduction in volume was seen at the end of the sampling period in tests carried out with solutions containing less than 50 μg mL^{-1} of zinc. A correction for water reabsorption (Martin-Villodre et al 1986) was made before calculation of kinetic parameters.

The zero-time sample was not utilized for disappearance rate calculations to negate membrane adsorption effects.

Appraisal of luminal zinc excretion

Two series of independent experiments were designed (5 animals per series). In the first series, the rat gut preparation was used as indicated for absorption tests but, simultaneously, an incision was made in the neck and the jugular vein exposed. At time zero, the gut was filled with 10 mL of zinc-free buffered physiological saline and 1 mL of an isotonic buffer containing 1500 μg mL^{-1} of zinc, was slowly injected into the jugular vein over 1 min, to approximate the zinc incorporation into the body from a perfused solution. After 30 min the gut was emptied and sample taken and assayed for zinc content. In the second series, 1 mL of zinc-free buffered saline was injected via the jugular vein.

Analytical procedure

Measurement of zinc concentration in samples was by means of a Perkin-Elmer, Model 2380 atomic absorption spectrophotometer. Instrumental and gas-flow settings, and precise aspiration rates were established to optimize the signal and to minimize background noise: wavelength 213·9 nm, flame air/acetylene, lamp current 15 mA, air 4 bars, slit width 0·7 mm, acetylene 0·9 bars, aspiration rate 6 mL min^{-1}, nebulizer system flow spoiler. For samples containing less than 0·4 μg mL^{-1} zinc, impact bead was used as the nebulizer system (sensitivity: 50 ng mL^{-1}). Excellent linear plots relating absorbance and concentration were obtained as calibration curves from standard solutions of zinc acexamate. Coefficients of variation ranging from 0·76 to 4·80% were found.

Biological samples were centrifuged at 3000 rev min^{-1} (1000 g) to remove intestinal debris, and the supernatant was used for zinc assays; an aliquot of the nonperfused working solution was used as a zero-time sample. All samples were diluted with HEPES 5 mM buffer until a suitable concentration was obtained. Samples were then sequentially determined, from the most dilute to the most concentrated for each set. Deionized water was continuously aspirated between measurements.

Results

Zinc excretion studies

Zinc excretion tests gave, in all cases, negative results. No more zinc was detected in the perfusion solutions at 30 min than in standard, zinc-free blanks.

Zinc absorption studies

The zinc concentration in perfusate is summarized in Table 1 (normal animals) and Table 2 (zinc-depleted animals). A first-order absorption model was fitted to each set of data

Table 1. Luminal zinc concentrations in normal rats (means of 5 animals ± s.d.); zero-time values are the initial perfusion concentrations (A_i). Mean absorption rate pseudoconstants, k_a, fitting each set of data, as well as actual zero-time intercepts, A_o, and correlation coefficients found for apparent first-order kinetics, r, are also given.

Time (min)	Zinc concn in luminal perfusates ($\mu g\ mL^{-1}$)				
0	5	50	500	1000	1500
5	2·50 ± 0·43	31·1 ± 2·3	333·7 ± 53·8	648·0 ± 45·6	1002·5 ± 56·3
10	1·99 ± 0·36	25·3 ± 1·9	305·2 ± 50·1	602·1 ± 46·4	940·8 ± 63·2
15	1·55 ± 0·26	21·0 ± 1·8	269·1 ± 44·4	529·3 ± 52·7	859·9 ± 63·2
20	1·26 ± 0·26	17·6 ± 1·7	249·0 ± 37·2	479·0 ± 54·4	825·1 ± 66·6
25	1·03 ± 0·19	14·6 ± 1·5	200·7 ± 27·4	429·7 ± 47·9	763·7 ± 62·7
30	0·85 ± 0·24	12·2 ± 1·5	184·2 ± 24·0	390·7 ± 46·6	724·5 ± 65·6
$k_a\ (h^{-1})$	2·598	2·230	1·476	1·249	0·785
$A_o\ (\mu g\ mL^{-1})$	3·05	37·00	386·7	727·0	1065·5
r	0·999	0·999	0·991	0·998	0·997

Table 2. Luminal zinc concentrations in zinc-deficient rats (means of 5 animals ± s.d.); zero-time values are the initial perfusion concentrations, A_i. Mean absorption rate pseudoconstants, k_a, fitting each set of data, as well as actual zero-time intercepts, A_o, and correlation coefficients, r, found for apparent first-order kinetics, are also given.

Time (min)	Zinc concn in luminal perfusates ($\mu g\ mL^{-1}$)		
0	25	500	1000
5	15·67 ± 1·43	341·4 ± 18·8	765·9 ± 42·9
10	12·96 ± 1·34	309·2 ± 22·8	628·4 ± 30·2
15	10·47 ± 1·55	233·2 ± 19·8	504·5 ± 46·9
20	8·92 ± 1·26	193·4 ± 8·7	417·8 ± 47·4
25	7·16 ± 1·34	145·6 ± 13·0	325·0 ± 44·0
30	5·89 ± 1·22	128·9 ± 10·3	266·1 ± 36·0
$k_a\ (h^{-1})$	2·343	2·583	2·554
$A_o\ (\mu g\ mL^{-1})$	19·08	439·4	957·2
r	0·999	0·993	0·999

(Wagner 1979) to yield an apparent absorption rate constant (k_a) and a zero time concentration (A_o), also shown in the tables.

Discussion

Zinc excretion into the small intestine

Although zinc excretion into the intestine via biliary and pancreatic secretions and from the mucosal surface of the epithelial cells has been demonstrated (Methfessel & Spencer 1973; Evans et al 1979; Weigand & Kirchgessner 1980), we have shown in our experimental conditions that this will not influence the interpretation of data as the amounts of zinc in luminal solutions, initially zinc-free, was, in both cases, negligible and identical to those found in blank samples. This indicates that zinc excretion is insufficient to exercise measurable effects on zinc homeostasis (Flanagan et al 1983), or requires some time to become significant (Menard et al 1981; Webb & Cain 1982).

Mucosal zinc uptake

As assessed from literature data (Kakemi et al 1969; Doluisio et al 1970), two kinetic processes could be differentiated from the perfused solutions as far as the disappearance (or uptake) rate is concerned: solute binding on the intestinal mucosa, which may be a reversible binding of the compound by the intestinal wall protein residues, and apparent solute absorption i.e. the transference of the solute from luminal solution to the cytoplasm of the columnar cells and to plasma, across the intestinal lipoidal membrane. These two processes are simultaneous but very different in nature. While the latter is a constant, rate-limited mechanism, mucosal binding could be a physicochemical phenomenon which follows the law of mass action and is virtually instantaneous (Curry 1977). Accordingly, we have assumed that at the first experimental sampling time (5 min) the equilibrium in the binding process has been achieved, and:

$$\ln A_{ao} = n \cdot \ln A_o + \ln K \qquad (1)$$

The concentration remaining in each solution at zero time, A_o, can be directly seen from Tables 1 and 2, whereas A_{ao} values would be equal to the differences between the initially perfused concentrations, A_i (i.e. zero time values) and the effective ordinate intercepts, A_o.

The plot according to equation 1 for normal animals is shown in Fig. 2, and has the following expression:

$$\ln A_{ao} = 1{\cdot}081 \cdot \ln A_o - 0{\cdot}601 \qquad (r = 0{\cdot}996) \qquad (2)$$

that is, the amount of mucosal bound zinc accounts for about 30% of the initial amount perfused (28·8 ± 5·5).

For zinc-depleted animals the percentage of bound zinc was lower and, apparently, concentration-dependent (from 23·7 to 4·3 per cent as perfusion concentration increases). In

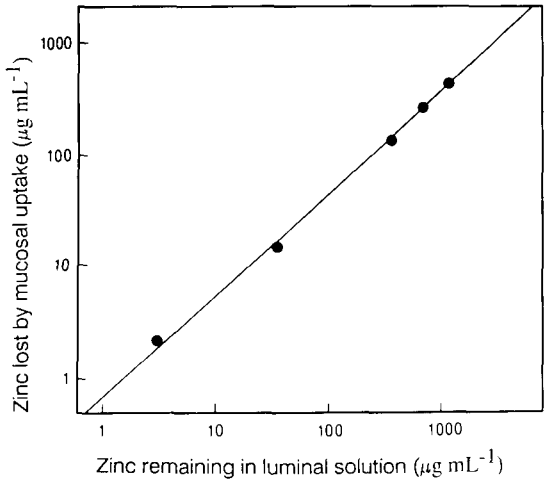

FIG. 2. Plot of mucosal-bound zinc vs zinc in solution in normal animals, according to equation 1, thus showing that mucosal binding is likely to be a purely physicochemical process.

the absence of indications of the effects of zinc deprivation on the integrity of biological absorbing membranes, the above results suggest that zinc mucosal binding in normal animals could be regarded as a reversible physicochemical process governed by adsorption laws, thus explaining the experimental observation of the presence of zinc in luminal perfusates when zinc-free isotonic media are perfused after an intestinal zinc loading (Hoadley et al 1987).

Apparent zinc absorption

We have called the process of net disappearance of zinc from solution when the binding process is completed, 'apparent zinc absorption'. The word 'absorption' is, therefore, used only as a functional expression describing the rate of zinc disappearance from luminal solution to some intracellular environment though the metal would be incorporated into the bloodstream, or transiently retained (Cousins 1986).

The apparent first order absorption rate constant, or pseudoconstant (k_a) for each animal was estimated by fitting equation

$$\ln A_s = -k_a \cdot t + \ln A_o \quad (3)$$

to data by linear regression. In equation 3, A_s represents the remaining zinc concentration in luminal samples at a given time t (i.e. from 5 to 30 min), and A_o is the theoretical luminal zinc concentration in the solution at zero time, which would not coincide with the experimental starting concentration because of the mucosal binding of the solute, as described above.

The average apparent first order rate constants found at each initial perfusion concentration were statistically compared by means of an ANOVA test and subsequent Ryan, Newman-Kuehl and Peritz F-tests (Harper 1984), for normal and for zinc-depleted rats.

Table 3. Statistical comparison between apparent k_a values found at different initial perfusion concentrations in the two groups of animals using the selected procedures (Harper 1984).

Tested group	Compared zinc initial perfusion concns ($\mu g\ mL^{-1}$)	Statistical significance (P values)
Normal animals	5 vs 50	0·0033
	500 vs 1000	0·0333
	1000 vs 1500	0·0080
	All other combinations	< 0·0001
Zinc-depleted animals	25 vs 500	0·649 (NS)
	25 vs 1000	0·482 (NS)
	500 vs 1000	0·932 (NS)

NS = Not significant.

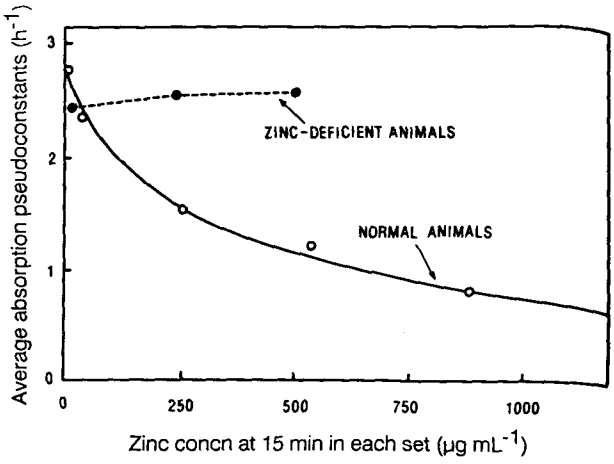

FIG. 3. Plots representing fitting of absorption rate pseudo-constants, k_a, found for each set of data, against corrected zinc concentrations at 15 min in luminal fluid. For normal animals, the hyperbolic-like sequence found would be indicative of Michaelis-Menten absorption kinetics.

Normal animals. In Table 3, k_a values show a statistically significant decrease as the initial perfusion concentration increases, demonstrating that the zinc absorption process is nonlinear and suggesting that a saturable mechanism of absorption is involved, as shown in Fig. 3. Accordingly, Michaelis-Menten and combined Michaelis-Menten and first order kinetics equations (in their integrated forms, equations 4 and 5, respectively) (Wagner 1979; Gibaldi & Perrier 1982):

$$t = \frac{1}{V_m}\left(A_o - A_s + K_m \cdot \ln \frac{A_o}{A_s}\right) \quad (4)$$

$$t = \frac{1}{k_a \cdot K_m + V_m} K_m \left[\ln \frac{A_o}{A_s} + \frac{V_m}{k_a} \ln \frac{(A_o + K_m)k_a + V_m}{(A_s + K_m)k_a + V_m}\right] \quad (5)$$

were fitted to the average concentration data for all initial perfusion concentrations and sampling times (Table 1). These fits were compared with those found when first-order kinetics is applied to the same data in the same fashion:

$$t = \frac{\ln A_o - \ln A_s}{k_a} \quad (6)$$

Table 4. Parameter values (±s.d.) found after fitting the selected model equations to the data obtained in normal animals in a global form. Statistics for each fit (SS = squared sum of weighted residuals, AIC = Akaike information criterion, r = correlation coefficient between experimental and model-prediction values) are also indicated.

Working equation	Parameter values	SS	AIC	r
Michaelis-Menten (eqn 4)	V_m = 1·033 (±0·02) mg mL^{-1} h^{-1}			
	K_m = 0·416 (±0·01) mg mL^{-1}	0·0147	−112·6	0·9997
	A_o = 1·061 (±0·11) mg mL^{-1}			
First-order (eqn 6)	k_a = 1·644 (±0·13) h^{-1}	0·2621	−28·2	0·9901
	A_o = 1·337 (±0·57) mg mL^{-1}			
Combined kinetics (eqn 5)	V_m = 0·651 (±0·03) mg mL^{-1} h^{-1}			
	K_m = 0·295 (±0·01) mg mL^{-1}	0·0145	−111·0	0·9997
	k_a = 0·306 (±0·06) h^{-1}			
	A_o = 1·081 (±0·22) mg mL^{-1}			

Details concerning this fitting method have been previously reported (Merino et al 1989a, b; Sánchez-Picó et al 1989).

Three features of the fits were compared in order to select the best kinetic model: the correlation coefficient between experimental and model-predicted values (r), the squared sum of weighted residuals (SS), and the AIC value found after application of the Akaike information criterion (Akaike 1976). These global fits (Table 4) indicate that the apparent intestinal absorption of the metal from its acexamic acid salt in normal rats can be characterized as a specialized transport mechanism, with a substantial contribution of passive non-saturable diffusion. Although the AIC figures seem to indicate the true Michaelis-Menten kinetics (eqn 4) as slightly more probable , when the same degree of probability exists the Akaike test will select the model with a lesser number of parameters (Yamaoka et al 1981). Since AIC figures are of the same order and the sum of weighted residuals favours the selection of the combined Michaelis-Menten and first-order equation (eqn 5), the latter has been thought to better describe the apparent actual kinetics of the zinc absorption process, as shown in Fig. 4. This pattern is basically in accordance with the data reported for normal animals in recent papers (Hoadley et al 1987), at least in qualitative aspects. Those authors found that the intestinal uptake of ^{65}Zn is governed by combined kinetics, although parameter values are different from those reported here because of essential differences in the biological technique, as well as the expression of the results.

On the basis of the perfused volume (which was 10 mL), a V_m value of 6·51 mg h^{-1} and a K_m value of 2·96 mg were found. The non-saturable diffusion component, k_a, was 0.306 h^{-1}, which accounts for 12% of the total absorption rate in non-saturated conditions (i.e. perfusion at the lower concentration tested), and 39% in almost saturated conditions (i.e. perfusion at the higher concentration assayed), as can be deduced from the data shown in Table 1. The apparent first-order rate constant accounting for the specialized absorption component (V_m/K_m) was 2·200 h^{-1}, which characterizes the zinc transport system as a high-capacity mechanism, only somewhat less than values found for aminocephalosporins (Sánchez-Picó et al 1989) and for β-alanine (Merino et al 1989b), both compounds having been tested using similar absorption techniques.

Zinc deficient animals. As shown in Table 3, no significant differences could be assessed between the apparent absorption rate constants found for all perfusion concentrations (25, 500 and 1000 μg mL^{-1}). The absolute k_a values fall within the range of those found in normal rats at low perfusion concentrations. Statistical comparison shows that the absorption rate pseudoconstants found at 500 and 1000 μg mL^{-1} in zinc-depleted animals are different from those found in normal rats at the same perfusion concentrations ($P < 0.0001$ in both cases). The conclusion is that the apparent zinc absorption is virtually non-saturated in this group of animals and, therefore, it cannot be characterized as a specialized process using these data alone (Fig. 3).

This experimental evidence tends to support several observations which postulate a homeostatic regulation of zinc absorption (Smith & Cousins 1980; Weigand & Kirchgessner 1980; Jackson et al 1981; Flanagan et al 1983; Cousins 1986). Simply speaking, a tendency of the body to palliate zinc deficiency seems to exist, although the ultimate cause of this phenomenon remains obscure and deserves further research.

Allometric considerations and practical implications

A K_m value of 295 μg mL^{-1} was obtained for the apparent absorption process according to equation 5 (Table 4), which, in terms of zinc amounts in the luminal fluid, is equal to 2·96 mg for a 350 g rat. If we assume that equation 5 virtually collapses to a linear first-order equation when approximately 0·3 K_m remains at the absorption site, we have: $295 \times 0{\cdot}3 = 88{\cdot}7$ μg mL^{-1} (see Fig. 4), that is, about 0·887 mg per rat. This could be considered the maximum dose below which linear kinetics should be maintained. Let us call this dose, D_r.

On the basis of allometric considerations, and even assuming the inaccuracies and severe limitations of such an extrapolation a predicted homologous dose of D_r in man, D_m, could be approximated (Peris-Ribera et al 1986; Sánchez-Picó et al 1989):

$$D_m = D_r(W_m/W_r)^{0.94} = 0.887\ (70/0{\cdot}35)^{0.94} = 129 \text{ mg}$$

In this expression, W_m and W_r represent the mean weights, in kg, assumed for man and rat. According to this expression, about 130 mg would be the limiting dose, over which nonlinear kinetics will be operative, giving rise to saturated absorption kinetics, in man.

Since in human therapy, usual maintenance doses of zinc acexamate range from 300 to 600 mg, or 48 to 96 mg of zinc per dose (Alcalá et al 1985; Varas 1986), it could be reasonably assumed that no saturable uptake of zinc can be expected in normal patients.

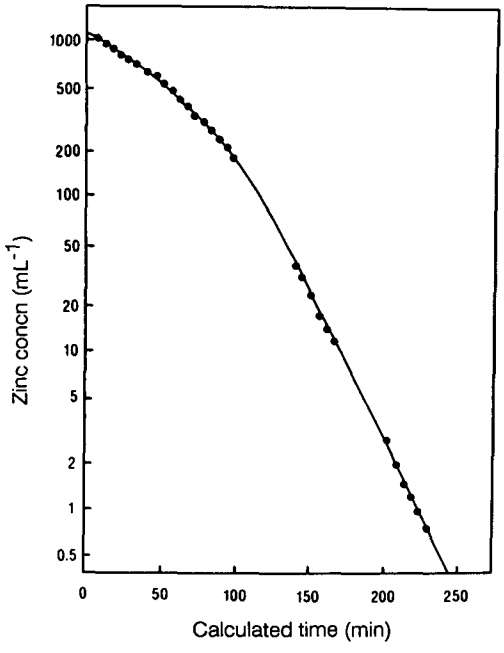

FIG. 4. Semilogarithmic continuous plot representing zinc absorption from the small intestine according to combined Michaelis-Menten and first-order kinetics (eq 5). Parameter values are shown in Table 4.

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References

Akaike, H. (1976) An information criterion (AIC). Math. Sci. 14: 5–9

Alcalá, R., Castellanos, D., Velo, J. L., González-Lara, V. (1985) Zinc acexamate in the treatment of duodenal ulcer. Lancet ii: 157

Alcock, N. (1981) The measurement of zinc in biological samples. Alcoholism 5: 355–356

Antonson, D. L., Barak, A. J., Vanderhoof, J. A. (1979) Determination of the site of zinc absorption in rat small intestine. J. Nutr. 109: 142–147

Cousins, R. J. (1986) Toward a molecular understanding of zinc metabolism. Clin. Physiol. Biochem. 4: 20–30

Curry, S. H. (1977) Drug Disposition and Pharmacokinetics. 2nd edn Blackwell Scientific Publ. London pp 78–91

Davies, N. T. (1980) Studies on the absorption of zinc by rat intestine. Br. J. Nutr. 43: 189–203

Dedrick, R. L. (1973) Animal scale-up. J. Pharmacokin. Biopharm. 1: 435–461

Doluisio, J. T., Billups, N. F., Dittert, L. W., Sugita, E. T., Swintosky, J. V. (1969) Drug absorption. I. An in situ rat gut technique yielding realistic absorption rates. J. Pharm. Sci. 58: 1196–1199

Doluisio, J. T., Crouthamel, W. G., Tan, G. H., Swintosky, J. V., Dittert, L. W. (1970) Drug absorption. III. Effect of membrane storage on the kinetics of drug absorption. Ibid. 59: 72–76

Evans, G. W., Johnson, E. C., Johnson, P. E. (1979) Zinc absorption in the rat determined by radioisotope dilution. J. Nutr. 109: 1258–1264

Faraji, B., Swendseid, M. E. (1983) Growth rate, tissue zinc levels and activities of selected enzymes in rats fed a zinc-deficient diet by gastric tube. Ibid. 113: 447–455

Flanagan, P. R., Haist, J., Valberg, L. S. (1983) Zinc absorption, intraluminal zinc and intestinal methallothionein levels in zinc-deficient and zinc-replete rodents. Ibid. 113: 962–972

Frommer, D. J. (1975) The healing of gastric ulcers by zinc sulphate. Med. J. Austral. 2: 793–795

Garrigues, T. M., Martin, U., Plá-Delfina, J. M., Prescott, L. F. (1990) Dose-dependent cefadroxil absorption and elimination. Paper read at the British Pharmacological Society. London, January

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics. 2nd edn Marcel Dekker Inc. New York, pp 271–318

Harper, J. F. (1984) Peritz F-test: basic program of a robust, multiple comparison test for statistical analysis of all differences among group means. Comput. Biol. Med. 14: 437–445

Hoadley, J. E., Leinart, A. S., Cousins, R. J. (1987) Kinetic analysis of zinc uptake and serosal transfer by vascularly perfused rat intestine. Am. J. Physiol. 252: G825–G831

Jackson, M. J., Jones, D. A., Edwards, R. H. T. (1981) Zinc absorption in the rat. Br. J. Nutr. 46: 15–27

Kakemi, K., Arita, T., Hori, R., Konishi, R., Nishimura, K. (1969) Absorption and excretion of drugs. XXVIII. The correlation between the absorption of barbituric acid derivatives from the rat small intestine and their binding to the mucosa. Chem. Pharm. Bull. 17: 248–254

Kowarski, S., Blair-Stanek, C. S., Schachter, D. (1974) Active transport of zinc and indentification of zinc-binding protein in rat jejunal mucosa. Am. J. Physiol. 226: 401–407

Martin-Villodre, A., Plá-Delfina, J. M., Moreno, J., Pérez-Buendia, M. D., Miralles, J., Collado, E. F., Sánchez-Moyano, E., Del Pozo, A. (1986) Studies on the reliability of a bihyperbolic functional absorption model. I. Ring-substituted anilines. J. Pharmacokin. Biopharm. 14: 615–633

Menard, M. P., McCormick, C. C., Cousins, R. J. (1981) Regulation of intestinal methallothionein biosynthesis in rats by dietary zinc. J. Nutr. 111: 1353–1361

Merino, M., Peris-Ribera, J. E., Torres-Molina, F., Sánchez-Picó, A., Garcia-Carbonell, M. C., Casabó, V. G., Martin-Villodre, A., Plá-Delfina, J. M. (1989a) Evidence of a specialized transport mechanism for the intestinal absorption of baclofen. Biopharm. Drug. Dispos. 10: 279–297

Merino, M., Polache, A., Peris-Ribera, J. E., Moreno-Dalmau, J., Plá-Delfina, J. M. (1989b) Absorption kinetics of beta-alanine as model compound in rat small intestine. Biochim. Biophys. Acta 986: 1–71

Methfessel, A. H., Spencer, H. (1973) Zinc metabolism in the rat. II. Secretion of zinc into the intestine. J. Appl. Physiol. 34: 63–67

Oestreicher, P., Cousins, R. J. (1982) Influence of intraluminal constituents on zinc absorption by isolated vascularly perfused rat intestine. J. Nutr. 112: 1978–1982

Peris-Ribera, J. E., Torres-Molina, F., García-Carbonell, M. C., Merino, M., Plá-Delfina, J. M. (1986) Absorción activa y alometria: possibles relaciones aplicables a los modelos animales de biodisponibilidad. Cienc. Ind. Farm. 5: 282–288

Sánchez-Picó, A., Peris-Ribera, J. E., Toledano, C., Torres-Molina, F., Casabó, V. G., Martín-Villodre, A., Plá-Delfina, J. M. (1989) Nonlinear intestinal absorption kinetics of cefadroxil in the rat. J. Pharm. Pharmacol. 41: 179–185

Sandstead, H. H., Vo-Khactu, K. P., Solomons, N. (1976) Conditioned zinc deficiences. In: Prasad, A. S. (ed.) Trace Elements in Human Health and Disease. Vol. 1. Academic Press, Inc., New York, pp 33–49

Seal, C. J., Heaton, F. W. (1983) Chemical factors affecting intestinal absorption of zinc, in vitro and in vivo. Br. J. Nutr. 50: 317–324

Seal, C. J., Heaton, F. W. (1987) Zinc transfer among proteins in rat duodenum mucosa. Ann. Nutr. Metab. 31: 55–60

Smith, J. C., Butrimovitz, G. P., Purdy, W. C. (1979) Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clin. Chem. 25: 1487–1491

Smith, K. T., Cousins, R. J. (1980) Quantitative aspects of zinc absorption by isolated vascularly perfused rat intestine. J. Nutr. 110: 316–323

Steel, L., Cousins, R. J. (1985) Kinetics of zinc absorption by luminally and vascularly perfused rat intestine. Am. J. Physiol. 248: G46–G53

Varas, M. J. (1986) Zinc acexamate and ranitidine in the short and mid term management of gastroduodenal ulcers; an evaluation of relapse rates. Curr. Ther. Res. 39: 19–29

Wagner, J. G. (1979) Fundamentals of Clinical Pharmacokinetics, 2nd edn, Drug Intelligence Publ. Hamilton, pp 247–284

Webb, M., Cain, K. (1982) Functions of methallothionein. Biochem. Pharmacol. 31: 137–142

Weigand, E., Kirchgessner, M. (1980) Total true efficiency of zinc utilization determination and homeostatic dependence upon zinc supply status in young rats. J. Nutr. 110: 469–480

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (Multi) for microcomputer. J. Pharm. Dyn. 4: 879–885