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Trace Metal Requirements in Total Parenteral Nutrition. Part 4. A Potentiometric Study of the Metal–Ligand Interactions in the Zinc–phenylalanine, Zinc–arginine, Zinc–phenylalanine–cysteine, Zinc–phenylalanine–histidine, Zinc–arginine–cysteine and Zinc–arginine–histidine Systems under Physiological Conditions, and Consequent Evaluation of the Optimal Dose of Zinc to be Added to a Nutritive Solution of a Known Composition

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Among other trace elements, zinc, copper and manganese are essential to human life. However, no precise data concerning the quantitative requirements of these metals during total parenteral nutrition (TPN) were available so far, although clinical studies had pointed out the specific role of TPN in the induction of excessive urinary excretions of copper and, first of all, zinc. Furthermore, no experimental method could be used to estimate metal doses likely to compensate for such extra-losses.

After the previous computer-based confirmation that the enhancement of the urinary excretions of zinc due to TPN closely depended on the very composition of the nutritive solution infused, a theoretical approach was developed by one of us, aiming at the determination of optimal metal doses as a function of each corresponding composition. Yet, the reliability of such calculated doses was proved to be dependent on that of the stability constants of the metal-amino acid complexes which compose the simulation model of the solution studied. A number of formation constant determinations had thus to be carried out under the appropriate physiological conditions. Some of them were already reported in the previous papers of this series.

The present work deals with (i) the ultimate determinations which bring the percentage of the experimentallly-based zinc complex concentrations over the satisfactory level of 80% for the nutritive mixture under study, (ii) the simulations which yield the optimal daily dose of zinc to be added to this given mixture. Attention is particularly drawn to the versatility of this method with a view to its application to every nutritive solution of a known composition.

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Introduction

During the past decade, the necessity of introducing trace metal ions into the solutions used in the artificial feeding technique known as total parenteral nutrition (TPN) has progressively emerged [1, 2]. This was due (i) to the recent discoveries concerning the essentiality of a growing number of trace elements in normal human diets [3–6], (ii) to the parallel clinical observations of the specific part played by TPN itself in the promotion of excessive trace metal excretions [7–12].

Among the trace metals which were taken into account in these nutrition problems, particular attention was drawn to zinc, which was incorporated in the 1974 issue of the U.S. National Academy of Science tables of 'Recommended Dietary Allowances' [13]. Indeed, zinc has been characterized as the essential cofactor of more than 80 metalloenzymes [3, 5] and its determining role in protein and nucleic acid biosynthesis makes it a crucial agent in wound healing and, more generally, in tissue repair [5]. This factor may be of some importance for people receiving TPN, as many of them follow the artificial nutrition route after surgical interventions on gastrointestinal organs [14]. In addition to this first incentive to supplement TPN patients with zinc, a more pressing need arose from the observation that extraordinary urinary losses of zinc were induced by the very administration of TPN solutions [1, 8, 9, 12].

Several attempts were made to estimate the ideal quantities of zinc likely to compensate for such abnormal losses [9, 10, 12], but all of them were empirical, actually requiring the determinations of the metal balances respective to each patient for each nutritive mixture. Indeed, it had been foreboded on the basis of clinical studies carried out on animals

[15–17] and man [18], that the excreted amounts of zinc were directly dependent on the composition of the nutritive solution administered [19].

This was confirmed by computer simulation in our first paper on this topic [20], in which a correlation was established between the enhancement of zinc urinary excretion and the proportions of cysteine and histidine in the composition of the solution. Hence, a theoretical approach was proposed, with a view to estimating the optimal doses of trace metals to be added to nutritive solutions, in order to compensate for the induced extra-losses [20, 21].

This approach was based on the principle that the nutritive solution should contain such a total concentration of metal that the corresponding free concentration be the same as that occurring in normal blood plasma. Its reliability therefore depended on the reliability of (i) the assessment of these free concentrations, which were derived from the available quantitative data on the interactions of each metal with the main plasma proteins [22], (ii) the stability constants of the complexes formed by the metals with the ligands present in the nutritive mixtures, a great number of which were to be determined under the appropriate experimental conditions.

Decision was first taken to test the applicability of the above mentioned approach to establish zinc, copper and manganese doses to be added to a nutritive mixture of a known composition. Calcium and magnesium, whose usual requirements were relatively well known, were incorporated to the simulation model as a particular test of validity. Quite acceptable results were obtained from this first estimation [20, 23], which was thus proved worth improving by the required stability constant determinations. It was then postulated that a realistic level of confidence for the metal ion equilibria in the nutritive mixture would be reached when the proportion of complex concentrations experimentally-based would represent about 95% of the original distribution.

Two series of such determinations have already been performed, involving successively cysteine, histidine and their zinc ternary complexes with glycine, lysine and threonine [24, 25]. The present paper deals with: (i) the determination of the formation constants in the zinc-phenylalanine and zinc-arginine binary systems, as well as the ternary systems resulting from the combination of each of the latter with cysteine and histidine, (ii) the computer-based estimation of the optimal dose of zinc to be added to the TPN solution under study. The following discussion will emphasize the applicability of the proposed method to any nutritive mixture of a known composition.

Formation Constant Determinations

Experimental

Products

All the amino acids were supplied by Merck as 'biochemical grade' products. They were systematically titrated before use, by means of classical Gran plots; they were thus employed without further purification. Their stock solutions were constantly kept under a nitrogen atmosphere. In spite of this, fresh cysteine solutions had to be prepared frequently, whenever a slight white precipitate appeared at the bottom of the containing flask.

The preparation of the stock solutions of zinc perchlorate (Pierce Inorganics B.V.), as well as their titration procedures, were already described in a previous paper of this series [24]. Sodium hydroxide solutions were also prepared and titrated in accordance with the specifications given in this paper [24], the initial concentrated volumetric solutions being supplied by BDH.

Merck reagent grade sodium perchlorate 0.15 mol dm^{-3} was used as the background salt to maintain activity coefficients constant, this concentration being isotonic with blood plasma. For each experiment, 'Normatom grade' Prolabo R.P. perchloric acid was used to set low initial pH values, so that all the donor groups of the ligands under study were initially protonated at the beginning of the titration.

Apparatus and technique

The potentiometric equipment, based on a digital voltmeter Beckman Model 4500, was identical to that mentioned in the previous study quoted above [24]. The electrode arrangements were also identical. It is only worthwhile to recall that the electrode system was calibrated in terms of concentrations, *i.e.* by determination of formal potentials using readings from solutions of known concentrations of hydrogen ions. As a consequence, the pH symbol that will be used throughout this study must be understood in terms of $-\log[H^+]$.

The initial total concentrations of the reactants used in the titrations are reported in Table I, along wih the corresponding pH ranges. The experiments were stopped whenever a precipitate appeared in the solution, as indicated by a steady drift in the mV-meter readings.

Calculation procedures

The MINIQUAD programme [26] was used to refine the approximate values initially assigned to the formation constants of the complexes potentially formed in a given system. The discrimination of the complex stoichiometries and the rough estimation of the corresponding formation constants were based

TABLE I. Summary of the Titration Data Used in the Formation Constant Calculations. Initial concentrations of zinc (C_{Zn}), first ligand (C_L), second ligand (C_X), strong acid (C_H) and pH* range. The concentrations are expressed in mmol dm^{-3}.

System	C_{Zn}	C_L	C_X	C_H	pH range
Proton-arginine		10.00		24.87	2.0–9.8
		10.00		39.80	1.6–9.9
		5.00		9.95	2.4–9.8
Zinc-phenylalanine	10.15	10.00		20.92	1.8–6.9
	5.07	10.00		25.37	1.7–7.0
	5.07	20.00		25.37	1.9–6.4
	2.03	20.00		25.06	1.9–7.1
	1.01	20.00		24.95	1.9–8.2
Zinc-arginine	10.15	10.00		25.91	1.9–6.9
	5.07	10.00		25.39	2.0–7.2
	20.30	10.00		21.97	2.1–6.6
	5.07	20.00		25.39	2.7–8.1
	7.10	20.00		25.60	2.7–7.9
	2.03	20.00		25.08	2.7–8.7
Zinc-phenylalanine-histidine	10.15	10.00	10.00	20.88	1.9–7.6
	10.15	20.00	10.00	30.88	2.1–5.7
	10.15	10.00	20.00	30.88	2.0–9.4
	5.07	5.00	5.00	15.45	2.2–7.2
	20.30	10.00	10.00	31.93	1.9–7.0
Zinc-phenylalanine-cysteine	10.15	10.00	10.00	30.88	1.7–5.1
	10.15	20.00	10.00	25.91	2.0–5.0
	5.07	5.00	5.00	20.42	1.8–5.4
	5.07	5.00	10.00	25.39	1.8–9.6
	5.07	10.00	5.00	25.39	1.8–8.6
	3.04	3.00	3.00	15.23	1.9–5.9
	3.04	6.00	3.00	15.23	2.0–9.0
	3.04	3.00	6.00	15.23	2.0–9.3
Zinc-arginine-histidine	10.15	10.00	10.00	30.43	2.3–7.3
	10.15	20.00	10.00	40.23	2.5–7.8
	10.15	10.00	20.00	40.23	2.4–9.5
	5.07	5.00	5.00	15.22	2.5–7.4
	20.30	10.00	10.00	31.48	2.3–6.9
Zinc-arginine-cysteine	5.07	5.00	5.00	20.12	2.0–5.5
	5.07	5.00	10.00	25.02	1.9–9.4
	5.07	10.00	5.00	25.02	2.1–5.4
	3.04	3.02	3.00	15.01	2.0–5.8
	3.04	6.00	3.00	15.01	2.2–8.4
	3.04	3.00	6.00	15.01	2.1–9.9

*As defined in the text.

on several considerations, depending on the type of system.

For binary complexation studies, the examination of the shape of the formation curves was essential to the guessing of the nature of the possibly existing species, a set of superimposable plots of the experimental values of $\bar{p}$ versus $-\log [L]$ being characteristic of the formation of simple and mononuclear species only. Indeed, the experimental definition of $\bar{p}$, as is shown by equation [1], does not imply any peculiarity regarding the metal complex ceomposition of the system [27].

$$\bar{p} = \frac{C_L - [L] - [LH] - [LH_2] - \ldots - [LH_s]}{C_M} \quad (1)$$

TABLE II. Stability Constants $\beta_{pqrs} = [M_rL_pX_qH_s]/[M]^r[L]^p[X]^q[H]^s$ of Parent Complexes at 37 °C and I = 0.15 mol dm^{-3} $NaClO_4$, as Used in the Related Calculations.

System	p	q	r	s	log β	Ref.
Proton-hydroxyde	0	0	0	−1	−13.38	27
Zinc-hydroxide	0	0	1	−1	−9.03	47
Proton-histidine	1	0	0	1	8.770	24
	1	0	0	2	14.643	
	1	0	0	3	16.400	
Zinc-histidine	1	0	1	0	6.336	24
	2	0	1	0	11.599	
	1	0	1	1	10.718	
	2	0	1	1	16.919	
Proton-cysteine	1	0	0	1	10.110	27
	1	0	0	2	18.078	
	1	0	0	3	20.050	
Zinc-cysteine	2	0	1	0	17.905	27
	1	0	1	1	14.604	
	2	0	1	1	24.114	
	4	0	3	0	42.278	
	4	0	3	1	48.313	
	4	0	3	2	54.082	
Proton-phenylalanine	1	0	0	1	8.775	48
	1	0	0	2	10.969	

In this equation, C_L and C_M represent the respective total concentrations of ligand and metal in the solution.

The estimation of the preliminary constants to be refined in the binary systems was then graphically derived from the quantitative features of the formation curves. For protonation constants, the approach was similar. In the ternary systems instead, the assessment of the formation constants to be refined was established mainly on statistical considerations, as generally expressed by the equation

$$\log \beta_{MLXH_s} = \tfrac{1}{2}(\log \beta_{ML_2H_s} + \log \beta_{MX_2H_s} + \log 4) \quad (2)$$

which is derived from the well known [28] relation

$$\log \beta_{MLX} = \tfrac{1}{2}(\log \beta_{ML_2} + \log \beta_{MX_2} + \log 4) \quad (3)$$

The results of the MINIQUAD calculations were first tested on numerical grounds (sum of squares residuals, R factor), but the final choice was made on the basis of graphical comparisons between experimental curves and their simulations, as obtained with the help of the PSEUDOPLOT programme [29]. For binary systems, formation curves of the type defined in equation (1) were compared. For ternary systems, we used our modified version of the PSEUDOPLOT programme [24], which allows the calculation of the average protonation number $\bar{s}$ of both of the ligands involved, through the equation below

$$\bar{s} = \frac{C_H + NDP_L \times C_L + NDP_X \times C_X - C_{OH} + [OH] - [H]}{C_L + C_X} \quad (4)$$

The symbols C_L, C_X, C_H and C_{OH} respectively represent the total concentrations of the first ligand L, second ligand X, strong acid and alkali in the solution, NDP being the number of dissociable protons of the pertinent ligand.

In these refinements, some formation constants obtained from other studies of one of the present authors had to be used as constant parameters. They are given in Table II, along with the corresponding references.

Results

Proton-arginine

In addition to its alpha amino acid moiety, arginine contains a remote functional group, separated from the former by three carbon atoms. This group has a considerable proton affinity: its formation constant has been previously reported to lie within the range 11.5–13.2 [30, 31] which implies that the proton will not be easily displaced by metal complexation. Presumably for this reason, Hallman *et al.* [32] did not take this protonation step into consideration in their complexation studies. Indeed, taking the first protonation constant into account in the complex formation constant calculations would result in a loss of accuracy on the latter, as the value of this protonation constant corresponds to a pH range in which the glass electrode readings are not very reliable. Such a situation was already encountered in the case of the protonation of citrate [27]. We thus decided, in accordance with Hallman *et al.* [32], to consider the number of dissociable protons of arginine to be nil, this implying that the L denomination actually corresponds to the conventional indissociability of the protonated remote functional group.

As a consequence, only the protonation constants of the amino acid moiety are shown in Table III.

Zinc-phenylalanine

The formation curve of this system consisted of a set of superimposable drawings, whatever the metal to ligand ratios. The curve limits were characteristic of the formation of the two species ML and ML_2. The corresponding constants are shown in Table III.

TABLE III. Stability Constants Obtained from This Work. The formula of the general complex is $Zn_r(L)_p(X)_q(H)_s$. n = number of experimental observations used in the calculations, S = sum of squares of residuals.

System	p	q	r	s	log β	S	n
Proton-arginine	1	0	0	1	8.781 ± 0.003	0.468 E − 06	147
	1	0	0	2	10.805 ± 0.004		
Zinc-phenylalanine	1	0	1	0	4.208 ± 0.008	0.556 E − 07	56
	2	0	1	0	8.165 ± 0.010		
Zinc-arginine	1	0	1	0	4.074 ± 0.005	0.107 E − 05	187
	2	0	1	0	7.883 ± 0.006		
Zinc-phenylalanine-histidine	1	1	1	0	10.209 ± 0.016	0.284 E − 05	286
Zinc-phenylalanine-cysteine	1	1	1	0	13.110 ± 0.081	0.483 E − 06	137
Zinc-arginine-histidine	1	1	1	0	10.005 ± 0.019	0.301 E − 05	297
Zinc-arginine-cysteine	1	1	1	0	13.652 ± 0.090	0.402 E − 06	181
	1	1	1	1	19.999 ± 0.041		

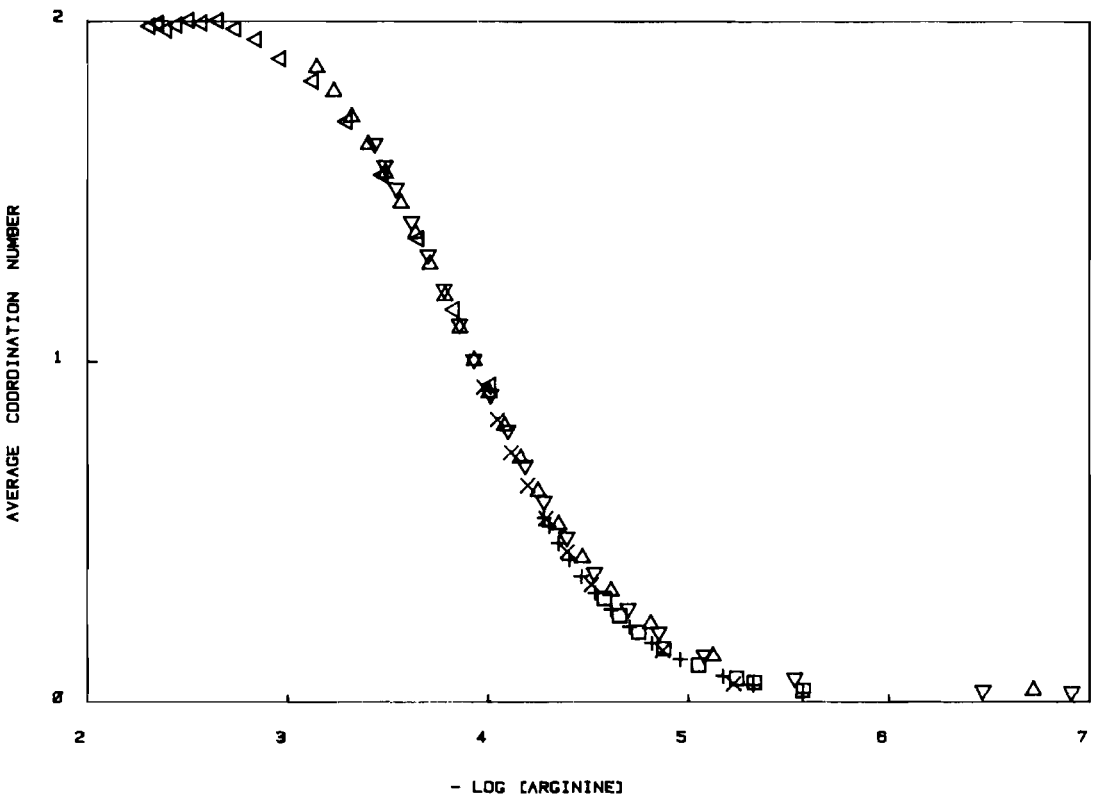

Fig. 1. Experimental formation curve of the zinc-arginine system. The symbols are in the respective order of the metal to ligand ratios given in Table I for the system under consideration: +, ×, □, △, ▽, ◁.

Zinc-arginine

The same type of formation curve as in the above system was obtained. Figure 1 shows the plots corresponding to the different metal to ligand ratios investigated. Once again, the ML and ML_2 complexes were expected to be formed, whose stability constants are given in Table III.

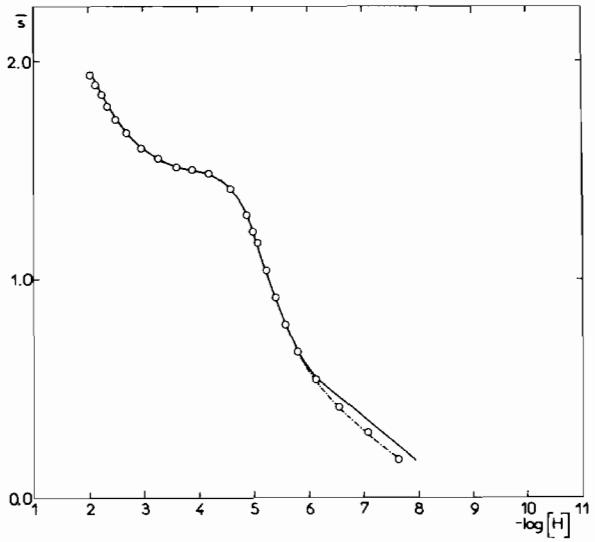

Fig. 2. Protonation curve of a mixture of phenylalanine and histidine in the presence of zinc: C_{Zn} = 10.15, C_{phe} = 10.00, C_{his} = 10.00 mmol dm^{-3}. Every fifth experimental point is materialized. The solid line simulates the curve assuming no mixed-ligand species formation. The broken line takes into account the ternary zinc-phenylalanine-histidine complex as given in Table III.

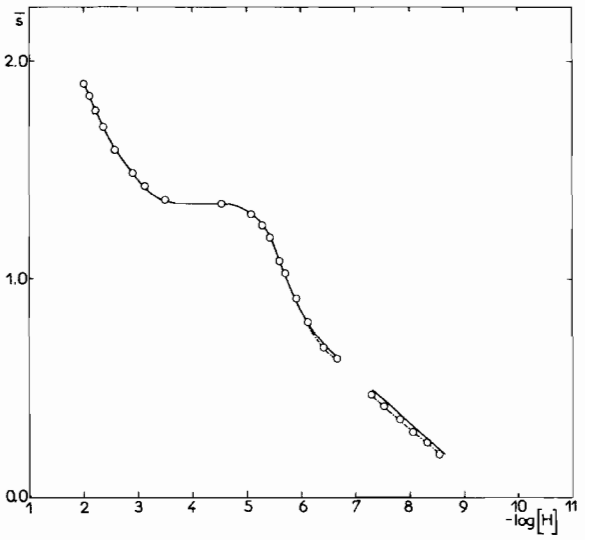

Fig. 3. Protonation curve of a mixture of phenylalanine and cysteine in the presence of zinc: C_{Zn} = 3.04, C_{phe} = 6.00, C_{cys} = 3.00 mmol dm^{-3}. Every fifth experimental point is materialized. The solid line simulates the curve assuming no mixed-ligand species formation. The broken line takes into account the ternary zinc-phenylalanine-cysteine complex as given in Table III.

It is noteworthy that, among other possibilities which were systematically tried anyway, the simultaneous MINIQUAD refinement of ML_2H with the first two species above lowered the sum of squares down to 0.700 E – 06. The respective constants were $\log \beta_{ML}$ = 4.032 ± 0.006, $\log \beta_{ML_2}$ = 7.919 ± 0.006, $\log \beta_{ML_2H}$ = 14.159 ± 0.047 in that case. However, in spite of the better numerical fit, this model was not finally selected because, (i) the standard deviations on the first two species were not improved, (ii) the PSEUDOPLOT simulations were not significantly different, even within the free ligand concentration ranges where ML_2H reached its maximum percentage of 10%, (iii) the examination of the protonation curves of arginine in the presence of zinc did not reveal any significant shift likely to account for the formation of such a protonated species [23].

Zinc-phenylalanine-histidine

The difference observed between the experimental protonation curve of the sum of the two ligands and its simulation assuming no mixed-ligand species (Fig. 2) shows that such species do exist. Accordingly, a stability constant was refined for the MLX complex, with a good accuracy (Table III). As is shown in Fig. 2, the introduction of this constant into the PSEUDOPLOT simulation resulted in a perfect coincidence with the experimental curves.

In addition to MLX, we tried to refine the constant of a possible MLXH species, but it was made negative by MINIQUAD.

Zinc-phenylalanine-cysteine

The ability of this system to form mixed-ligand complexes seems rather poor. As a matter of fact, the experimental protonation curves almost coincided with their simulations based on binary complexes only. Even the 1:2:1 ratio (Fig. 3), which is the most favourable to ternary complexation with regard to the high stability of the cysteine binary complexes, did not produce a notable difference. A MLX complex was nevertheless characterized (Table III), which yielded a very good graphical coincidence.

The MLXH constant which was then refined together with that of MLX did not become negative, but its introduction in the model did not improve the previous sum of squares and graphical fits either, and its percentage never went over 1%. It was thus considered to be negligible [23].

Zinc-arginine-histidine

The behaviour of this system was quite similar to that observed for the above zinc-phenylalanine-histidine one. A constant was refined for MLX (Table III), which allowed a good coincidence between the experimental protonation curves of the ligands and

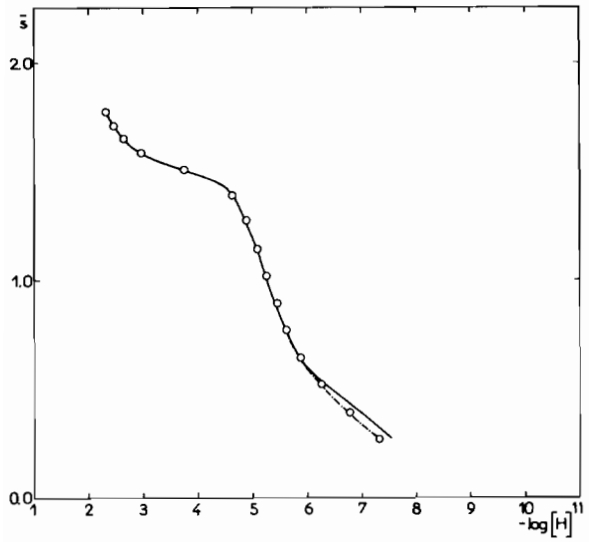

Fig. 4. Protonation curve of a mixture of arginine and histidine in the presence of zinc: C_{Zn} = 10.15, C_{arg} = 10.00, C_{his} = 10.00 mmol dm^{-3}. Every fifth experimental point is materialized. The solid line simulates the curve assuming no mixed-ligand species formation. The broken line takes into account the ternary zinc-arginine-histidine complex as given in Table III.

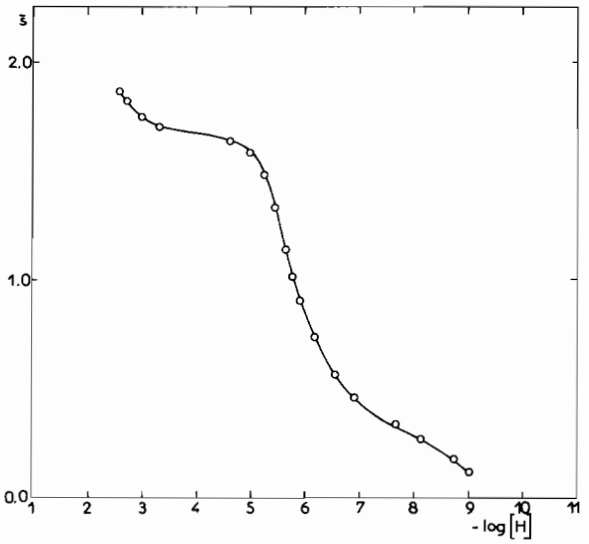

Fig. 5. Protonation curve of a mixture of arginine and cysteine in the presence of zinc: C_{Zn} = 3.04, C_{arg} = 3.00, C_{cys} = 6.00 mmol dm^{-3}. Every fifth experimental point is materialized. The solid line simulates the curve assuming no mixed-ligand species formation.

their simulations when taking this mixed-ligand species into account (Fig. 4).

A possible MLXH complex was introduced in the model together with MLX. The relevant constant was not made negative, but its presence did not improve the previous sum of squares, the graphical fits or the standard deviation of the MLX constant. Moreover its maximum percentage reached only 1.6%. It was thus discarded as being negligible.

Zinc-arginine-cysteine

The study of this system was substantially limited by the poor solubility of some of the involved species, especially whenever the arginine concentrations were equal or superior to those of cysteine. The example given in Fig. 5 shows that the use of high cysteine concentration ratios allows the investigation of larger pH intervals, but, as a counterpart, prevents the formation of significant mixed-ligand complexes, this being due to the great zinc avidity of cysteine on its own.

Two possibilities were offered in this system, among which the discrimination was particularly difficult.

MLX was first refined alone: we obtained $\log\beta_{MLX}$ = 14.125 ± 0.036, with S = 0.604 E − 06. Then, the simultaneous refinement of MLX and MLXH yielded the results given in Table III. Some doubt subsists as to the choice of the "best" set in the present system, due to the fact that the introduction of MLXH in the refinement did not improve the standard deviation of the MLX constant. However, we finally chose the results in Table III because the sum of squares was found better in that case, and the graphical fits were slightly improved [23]. Finally, it is worth mentioning that, except a few points in the basic pH range (after redissolution of the precipitate) for which MLX reached more than 70%, MLX and MLXH never went respectively over 7% and 12% when refined together, whereas MLX was never superior to 14% when refined by itself.

Discussion

Binary systems

Few studies had so far been devoted to the zinc-phenylalanine system, two of them [33, 34] being carried out under ionic strength conditions similar to those used throughout the present work, but at 25 °C. This difference of temperature can account for the fact that our stability constants in Table III were found significantly lower than those (respectively 4.50 and 8.36 for ML and ML_2) calculated by Giroux and Henkin [33]. By contrast, the values mentioned by Demaret *et al.* [34] seem surprisingly low (4.06 and 7.93), as compared with other values determined at 25 °C (references quoted in ref. 34) and, first of all, with ours.

Two groups of authors had previously studied the zinc-arginine system [30, 32]. Clarke and Martell

TABLE IV. Increments of Stability for the Formation of Mixed-Ligand Complexes of Zinc and Histidine, or Cysteine, with Various Amino Acids.

System	$\Delta \log \beta$	$\Delta \log K$	Ref.	Experimental conditions
Zinc-histidine-cysteine	0.034	0.154	24	37°C, $NaClO_4$ 0.15 *M*
	0.10	–	32	37°C, KNO_3 0.15 *M*
Zinc-glycine-histidine	0.058	−0.545	24	37°C, $NaClO_4$ 0.15 *M*
	0.16	−0.26	37	25°C, KCl 0.2 *M*
	0.225	−0.41	38	25°C, KNO_3 0.1 *M*
	0.345	−0.27	39	37°C, KNO_3 0.15 *M*
Zinc-glycine-cysteine	−1.735	−1.445	24	37°C, $NaClO_4$ 0.15 *M*
Zinc-threonine-histidine	−0.376	−0.940	25	37°C, $NaClO_4$ 0.15 *M*
Zinc-phenylalanine-histidine	0.027	−0.335	This work	
Zinc-phenylalanine-cysteine	−0.229	0.302	This work	
Zinc-arginine-histidine	−0.036	−0.405	This work	
Zinc-arginine-cysteine	0.454	0.978	This work	

[30], who took into account the first protonation step of arginine, considered the ligand to be LH when they mentioned the existence of MLH and $M(LH)_2$. On the contrary, Hallman *et al.* [32] neglected the first protonation step (see the paragraph above) and characterised the ML, ML_2, and MLOH species. As can be seen from Fig. 1, no deviation from the average formation curve was observed, which could be attributable to the formation of a hydroxo species. Regarding the stoichiometry of the complexes, our results thus confirm those found by Clarke and Martell. From the numerical point of view, our values for the ML and ML_2 constants are intermediate to those mentioned by Hallman *et al.* (4.03 and 7.56) and by Clarke and Martell (4.11 and 8.07). This is not in favour of Hallman *et al*'s results, for the experimental conditions they used were more similar to ours than those used by Clarke and Martell.

Ternary systems

No previous study had been performed on the ternary systems investigated in the present paper. No comparison with former values could thus be made. Nevertheless, it may be of interest to discuss the ability of the involved aminoacids to form mixed-ligand species with zinc.

This implies the comparison of the experimental ternary constants with the values derived from the statistical equation [28]:

$$\Delta \log\beta = \log\beta_{1110} - \tfrac{1}{2}(\log\beta_{2010} + \log\beta_{0210} + \log 4) \quad (5)$$

and

$$\Delta \log K = \log K^{ML}_{MLX} - \log K^{M}_{MX}$$

$$= \log K^{MX}_{MLX} - \log K^{M}_{ML}$$

$$= \log \beta_{1110} - \log\beta_{1010} - \log\beta_{0110} \quad (6)$$

The main advantage of equation (5) is to analyze the ability of two ligands to form ternary species on purely statistical grounds. Instead, that of equation (6) lies in the fact that only the corresponding binary 1:1 complexes contribute to its formulation [35].

These comparisons can be extended to all the ternary systems involved in the present series [24, 25], for the specific reason that all of them are based on the same zinc-histidine and zinc-cysteine parent systems.

Table IV collects the corresponding increments of stability, along with some derived from literature, when available on the same systems. From a general point of view, any negative value of $\log \beta$ is characteristic of a certain destabilisation with respect to the normal statistical stability of the mixed-ligand species under consideration. On the contrary, Δ log K is normally expected to be negative, as the bonding of the first ligand with the metal ion is usually easier than that of the second one; yet, this rule suffers a few exceptions, especially in the case of π back coordination with some donor atoms, like sulphur in cysteine for example [27].

Let us now examine the tendencies to mixed-ligand coordination revealed by the data in Table IV. Regarding the ternary systems based on zinc-histidine, it can be seen that, whatever the various contributions of the 1:1 binary complexes, the stability of the mixed species is of the order of the statistical expectations. The only exception is the zinc-threonine-histidine system; attention has already been drawn to the destabilisation of its ternary chelate [25], presumably due to ligand-ligand interactions [36].

Apart from the zinc-histidine-cysteine system itself, the results observed within the group of ternary systems based on zinc-cysteine are more dissimilar. The mixed-ligand coordination is, now clearly

TABLE V. Original and Final Distributions of Zn(II) among Its Predominant Complexes in the Nutritive Mixture at pH* = 7.40. In both distributions, the species which were investigated in this series are ticked.

a) Original distribution Species Composition				logβ	Percentage	b) Final distribution Species Composition				logβ	Percentage
HIS1(1)	GLY1(2)	ZN+2(1)	H +1(1)	23.10	34.8%	CYS2(1)	HIS1(1)	ZN+2(1)		15.09	26.9%
CYS2(1)	HIS1(2)	ZN+2(1)	H +1(1)	26.50	20.9%	HIS1(2)	ZN+2(1)			11.60	17.3%
CYS2(1)	HIS1(1)	ZN+2(1)		15.23	14.8%	CYS2(2)	ZN+2(1)			17.91	8.9%
HIS1(2)	ZN+2(1)			11.68	7.2%	CYS2(1)	HIS1(2)	ZN+2(1)	H +1(1)	25.54	7.0%
CYS2(2)	ZN+2(1)			17.91	4.1%	ARGO(1)	CYS2(1)	ZN+2(1)		13.65	4.5%
HIS1(1)	CYS2(1)	ZN+2(1)	H +1(1)	21.60	1.4%	HIS1(1)	CIS2(1)	ZN+2(1)	H +1(1)	19.55	2.6%
HIS1(2)	GLY1(1)	ZN+2(1)	H +1(1)	21.89	1.4%	GLY1(1)	HIS1(1)	ZN+2(1)		10.62	2.5%
GLY1(1)	HIS1(1)	ZN+2(1)		10.62	1.0%	CYS2(1)	GLY1(2)	ZN+2(1)	H +1(1)	24.75	2.1%
HIS1(1)	PHE1(1)	ZN+2(1)		10.37	0.9%	ARGO(1)	HIS1(1)	ZN+2(2)		10.01	2.0%
CYS2(1)	GLY1(1)	ZN+2(1)		13.81	0.9%	HIS1(1)	PHE1(1)	ZN+2(1)		10.21	1.7%
HIS1(1)	CIS2(1)	ZN+2(1)	H +1(1)	19.55	0.8%	HIS1(1)	CYS2(1)	ZN+2(1)	H +1(1)	21.23	1.5%
ARGO(1)	HIS1(1)	ZN+2(1)		9.97	0.7%	CYS2(1)	THR1(1)	ZN+2(1)		13.44	1.3%
CYS2(1)	PHE1(1)	ZN+2(1)		13.49	0.7%	HIS1(1)	LEU1(1)	ZN+2(1)		10.43	1.1%
LYS1(1)	HIS1(1)	ZN+2(1)	H +1(1)	20.59	0.6%	HIS1(1)	LYS1(1)	ZN+2(1)	H +1(1)	20.33	1.1%
ARGO(1)	CYS2(1)	ZN+2(1)		13.09	0.5%	HIS1(1)	VAL1(1)	ZN+2(1)		10.27	1.0%
HIS1(1)	THR1(1)	ZN+2(1)		10.26	0.5%	ASP2(1)	HIS1(1)	ZN+2(1)		10.80	0.9%
HIS1(1)	LEU1(1)	ZN+2(1)		10.47	0.5%	ALA1(1)	HIS1(1)	ZN+2(1)		10.43	0.8%
LYS1(1)	CYS2(1)	ZN+2(1)	H +1(1)	23.71	0.4%	CYS2(1)	LEU1(1)	ZN+2(1)		13.59	0.8%
ASP2(1)	HIS1(1)	ZN+2(1)		10.84	0.4%	CIS2(1)	HIS1(1)	ZN+2(1)		11.62	0.8%
CYS2(1)	THR1(1)	ZN+2(1)		13.38	0.4%	CYS2(1)	CIS2(1)	ZN+2(1)	H +1(1)	22.30	0.7%
HIS1(1)	VAL1(1)	ZN+2(1)		10.31	0.4%	CYS2(1)	PHE1(1)	ZN+2(2)		13.11	0.7%
CYS2(1)	LEU1(1)	ZN+2(1)		13.59	0.4%	CYS2(1)	VAL1(1)	ZN+2(1)		13.43	0.7%
ALA1(1)	HIS1(1)	ZN+2(1)		10.47	0.4%	GLU2(1)	HIS1(1)	ZN+2(1)		10.75	0.7%
ASP2(1)	CYS2(1)	ZN+2(1)		13.96	0.3%	HIS1(1)	THR1(1)	ZN+2(1)		9.86	0.7%
CYS2(1)	VAL1(1)	ZN+2(1)		13.43	0.3%	CIS2(1)	HIS1(1)	ZN+2(1)	H +1(2)	26.35	0.7%
GLU2(1)	HIS1(1)	ZN+2(1)		10.75	0.3%	ASP2(1)	CYS2(1)	ZN+2(1)		13.95	0.6%
ALA1(1)	CYS2(1)	ZN+2(1)		13.59	0.3%	ALA1(1)	CYS2(1)	ZN+2(1)		13.59	0.6%
CYS2(2)	ZN+2(1)	H+1(1)		24.11	0.3%	CYS2(2)	ZN+2(1)	H+1(1)		24.11	0.6%
CYS2(1)	CIS2(1)	ZN+2(1)	H +1(1)	22.30	0.3%	HIS1(1)	PRO1(1)	ZN+2(1)		11.10	0.4%
CIS2(1)	HIS1(1)	ZN+2(1)		11.62	0.2%	HIS1(1)	ZN+2(1)			6.34	0.4%
HIS1(1)	PRO1(1)	ZN+2(1)		11.14	0.2%	ARGO(1)	CYS2(1)	ZN+2(1)	H +1(1)	20.00	0.4%

*As defined in the text.

favoured, as is the case of arginine, now clearly hindered, especially in the case of glycine. This situation is all the more surprising since phenylalanine and arginine are glycine-like bonded to zinc. Significant ligand-ligand interactions are thus expected between cysteine and the remote guanido group of arginine, as well as, to a lesser extent, between cysteine and the phenyl group of phenylalanine.

Evaluation of the Optimal Dose of Zinc for a Nutritive Mixture of a Known Composition.

Computer Simulation of the Metal Ion Equilibria in the Nutritive Mixture

As has been outlined in the introduction, the first test of applicability of our approach to the problem of the metal supplementation during TPN [21] was based on the simulated distribution of the metal complexes in the nutritive mixture, as obtained from stability constants available in the literature at the moment, or derived from chemical or statistical considerations [20]. The corresponding daily dose of zinc amounted to nearly 40 mg [23].

A better assessment of this dose required an improvement of the reliability of the simulated distribution of zinc in the nutritive solution. A great number of zinc-amino acid ternary systems had therefore to be newly investigated or reexamined under the adequate experimental conditions. Yet, the 33 components of the solution that were taken into account did not give rise to less than 3400 individual species; clearly, it was of out of question to determine all the related formation constants. The degree of urgency of these determinations was thus taken in accordance with the order of the decreasing concentrations of the involved zinc complexes. The experimental control of at least 80% of the total zinc concentration was considered as an attainable level of confidence for the simulation model.

The results obtained from the subsequent experimental determinations [24, 25], as well as from the present work, were finally introduced in the simulation model based on the ECCLES program set up by May *et al.* [22]. The final detailed distribution is shown in Table V: there it can be compared with the aforementioned original one. The complexes which have been investigated in this series have been ticked: it is noteworthy that the sum of the concentrations of all the species pertaining to the corresponding systems did represent about 95% of the total concentration of zinc in the preliminary distribution.

Implications of the Complex Distribution on the Evaluation of the Daily Dose of Zinc to be Infused

The first important result that should be emphasized is that the daily dose of zinc, which had originally been evaluated near 40 mg, has now decreased down to 21 mg. Clearly, this results from: (i) the disappearance of the major species in the preliminary distribution (*i.e.* zinc-his-$(gly)_2$-H) which had been mentioned by Hallman *et al.* [32], but has since been proved not to exist [24], (ii) the decrease of stability for a large number of the complexes which were investigated throughout our successive studies [24, 25].

As far as the distribution of the zinc complexes itself is concerned, this tendency explains that the total proportion of the examined species has decreased from the original percentage of about 95% to the final one of about 83% only; this is actually due to the resulting multiplication of species representing approximately 1% of the total zinc only (see Table V).

However, the decrease of the total concentration of zinc has been somewhat compensated for by a parallel phenomenon which is worth mentioning here. The greater part of the proton-ionization constants of the amino acids involved in the original simulation was used as found by Perrin *et al.* [32, 40]. These constants are defined as *mixed* constants, as they express the *concentrations* of the ligands, but also the *activity* of the hydrogen ion [32]. For this reason, whenever a proton-ionization constant is determined as expressing the concentrations of all the reactants, like those appearing in our various works [24, 25, 27, 41, 42], it is found about 0.12 to 0.14 lower than that previously obtained by Perrin's school; this had already been pointed out in a recent paper of one of the present authors [42]. Concerning the distribution under study, this has resulted in the slight increase of all the free amino acid concentrations, hence in the corresponding enhancement of the concentrations of all the complexes in which they take part. This antagonized to some extent the sharp decrease in the zinc concentration evoked above.

Discussion of the Proposed Dose of Zinc

The principle on which the calculation of the dose of zinc is based is aiming at maintaining the plasma level of zinc at its normal value, in such a way that the zinc amount infused with the nutritive solution be urinarily excreted instead of that excreted at the expense of the patient's body stores in the absence of zinc supplementation.

This means that this approach does not take into account the possible metal losses arising from bowel diseases themselves, such as fistulae or protein-losing enteropathy. Moreover, by definition, trying to maintain the free zinc concentration in plasma at its normal level does not imply that the administration of the corresponding metal dose to a patient originally deficient in zinc would replenish his depleted stores.

Interesting data with which our proposed zinc dose could be compared were recently discussed by

Klevay *et al.* [4] for oral normal nutrition. It appears from the considerations developed by these authors, as well as by others [43], that the oral daily dose of 15 mg recommended by the U.S. Food and Nutrition Board [13], is certainly not excessive. Indeed, the concentration of zinc in sweat is about the same as in plasma [44], and constitutes an insensible loss in the metabolic balance studies from which the above estimation has been derived.

Regarding the case of the TPN patients, who have to suffer specific urinary losses due to the infusion of the nutritive mixture itself, it thus seems logical that the infused amount of zinc should be superior to the normal oral dose. This remark appears all the more valid as patients fed by intravenous route are particularly likely to be initially zinc deficient [5].

It must be kept in mind that the daily dose of zinc estimated in the present work is specific to the composition of the nutritive mixture under consideration [45, 20]. Nevertheless, the basic principle developed in this study can be extended to every solution of a known composition [46].

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