Histamine as a Ligand in Blood Plasma. Part 3.† Potentiometric Study and Simulated Distribution of the Zinc–Histamine Ternary Complexes with Cysteinate, Histidinate, Glutaminate, Threoninate, and Citrate

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Knowing about the distribution of the metal–histamine complexes in blood plasma may help to understand some of the interactions between metal ions and histamine which have been observed *in vivo*, particularly as far as the influence of zinc and copper is concerned on the physiological activity of this mediator. With this in mind, the present work deals with the experimental determination of the formation constants of the most predominant zinc–histamine ternary species, as predicted from a previous study. Accordingly, the ternary zinc–histamine systems formed with cysteinate, histidinate, glutaminate, threoninate, and citrate were investigated at 37 °C and I = 0.15 mol dm^{-3} Na[ClO_4]. Interpretations derived from the simulated distribution of these complexes in the presence of the interfering copper–histamine–histidinate species are given, concerning a possible mode of action for zinc and copper towards histamine *in vivo*.

SEVERAL recent biological studies have revealed that, compared to other metal ions, zinc is particularly efficient in inhibiting histamine release from mast cells, either by itself [1] or through its complexes with anaesthetics.[2] In the latter case the mode of action of zinc has been attributed to a membrane phenomenon.[3,4]

Apart from this important property, developed as far as preventing the very process of histamine release into the extracellular fluid, it has also been observed that zinc is able to counteract the physiological activity of neurotransmitter injected in the blood stream, whereas copper enhances it.[5] Therefore, the use of zinc may, in the future, help to prevent the various manifestations of allergic disorders by inhibiting release of allergic mediators. It seems that zinc could also be used to attenuate the pharmacological effects of histamine after its excessive release from mast cells. This kind of approach could be particularly helpful for treating anaphylactic and anaphylactoid shocks.

Before such an application can be envisaged, it is necessary to understand the way in which zinc and copper, which antagonize each other, may influence the metabolism of histamine. The main objective in understanding this process apparently lies in the knowledge of the metal–histamine complex distribution in normal blood plasma. Therefore, our first study on this topic was devoted to determining the equilibrium constants of the binary metal–histamine complexes under plasma conditions.[6] Thereupon, an approximate distribution of the metal–histamine complexes was obtained by means of a previous simulation model,[7] already used by one of us,[8] based on the statistically estimated corresponding ternary constants. In fact, this simulated distribution pointed out the predominance of some zinc and copper ternary complexes. Therefore an experimental check on the relevant stability constants became necessary.

Hence, subsequent to the study of some of the major ternary copper–histamine species,[9] we have investigated the estimated predominant zinc–histamine ternary systems [6] which are formed with cysteinate (CysO), histidinate (HisO), cystinate (CisO), glutaminate (GlnO), threoninate (ThrO), and citrate. After a general discussion about the ligands' ability to form ternary species, we have examined the effect of our results on the distribution of the complexes under consideration, in the presence of the most concentrated copper–histamine ternary complex,[6] *i.e.* copper–histamine–histidinate, with a view to interpreting the influence of zinc and copper on the physiological activity of histamine *in vivo*.

† Part 2 is ref. 9.

EXPERIMENTAL

Reagents.—The L-amino-acids were obtained from Merck (Biochemical grade) and citric acid from Prolabo R.P. (p.a.). All were kept under an atmosphere of dried nitrogen. Histamine, in the form of crystalline free base, was supplied in sealed ampoules by Sigma Chemical Co. The purity of the ligands was potentiometrically checked; all were then used without further purification.

Sodium hydroxide solutions were prepared by diluting the contents of B.D.H. concentrated volumetric solution vials with deionised freshly boiled water, under an atmosphere of nitrogen. They were standardised against potassium hydrogenphthalate (Prolabo R.P., p.a.) and from the features of the Gran titration plots [10] were proved to be carbonate-free.

Sodium perchlorate (Merck p.a.) solutions were prepared as previously described.[8] Stock solutions of zinc perchlorate (Pierce Inorganics B.V.) were standardised by complexometric titrations using Eriochrome Black T as an indicator.[11] They were made slightly acid with perchloric acid (Prolabo, Normatom grade) so as to prevent hydrolysis and absorption of carbon dioxide. Their mineral acid content was deduced from direct potentiometric readings.

Potentiometric Equipment.—Potentiometric titrations were carried out with cells of type (1) using a Beckman

Glass electrode|Ligands, Zn^{2+}, Na[ClO_4] (0.15 mol dm^{-3})||NaCl (saturated)|Hg_2Cl_2/Hg (1)

S39301 glass electrode and a saturated sodium chloride Ingold calomel electrode fitted in an Ingold cell system.

Measurements of e.m.f. were recorded by means of a Beckman model 4500 digital voltmeter, the reproducibility of which was 0.1 mV. For each titration, 20 cm^3 of solution were titrated against the standard sodium hydroxide solution delivered from an ABU 12 Radiometer Autoburette.

Experimental Conditions.—Each solution for titration was freshly prepared from known volumes of perchloric acid and zinc stock solutions, and a known weight of the investigated ligand. Sodium perchlorate was added at $I = 0.15$ mol dm^{-3} as an ionic background to hold activity coefficients constant and to ensure isotonicity with blood plasma.

The temperature was maintained at 37.00 ± 0.02 °C in the reaction cell by circulating thermostatted water. All the experiments were performed under an atmosphere of thermostatted, scrubbed, carbon dioxide- and oxygen-free nitrogen supplied by L'Air Liquide Co. (' U ' grade).

The electrode system (I) was calibrated in terms of hydrogen-ion concentrations. The value used for pK_W was 13.38, as previously determined by one of us.[8] Each titration in the presence of zinc was stopped when precipitation occurred in the solution, as indicated by a steady drift in the voltmeter readings.

Besides the study of the ternary systems mentioned above, we had also to determine the equilibrium constants for zinc with glutaminate and threoninate, in order to find evidence of ternary complex formation of the metal ion with histamine. Ranges of zinc concentrations, ligand concentrations, mineral acid concentrations, and −log [H] values used in calculating the constants are summarised in Table 1. All titration data have been deposited as Supplementary Publication No. SUP 22872 (36 pp.),* so the −log [H] ranges shown in Table 1 correspond to solutions in which complexation was proved to be significant during the following calculations. In the light of this, it is noteworthy that, as previously pointed out,[12] the zinc–cystinate system gave rise to precipitation as soon as complexation occurred and could not be investigated further.

TABLE 1

Summary of the titration data used in obtaining stability constants. Initial total concentrations of zinc (C_{Zn}), first ligand histamine (C_L), second ligand (C_X), strong acid (C_H), and −log [H] range

System	C_{Zn}	C_L	C_X	C_H	−log [H] range
	mmol dm^{-3}				
Zinc–glutaminate	5.07		20.00	20.54	4.3—8.2
	5.07		10.00	10.53	3.8—7.5
	10.15		5.00	6.04	4.9—6.9
	5.07		5.00	5.52	4.2—7.2
	10.15		10.00	11.05	4.2—7.0
Zinc–threoninate	5.07		20.00	32.77	2.1—8.1
	10.15		10.00	17.16	2.1—6.4
	5.07		10.00	22.02	3.4—7.3
	10.15		5.00	11.79	2.2—6.5
	5.07		5.00	11.27	3.8—7.0
Zinc–histamine–cysteinate	5.07	9.23	10.01	35.20	1.9—10.3
	5.07	9.23	5.00	25.30	2.3—8.9
	5.07	4.62	10.01	20.33	2.1—10.1
	5.07	4.62	5.07	15.38	2.3—5.5
	2.54	2.31	2.53	10.18	2.3—7.9
Zinc–histamine–histidinate	10.15	9.23	10.00	40.68	2.0—7.5
	5.07	9.23	10.00	40.16	2.0—9.6
	5.07	9.23	5.00	30.25	2.2—8.4
	5.07	4.62	10.00	25.30	2.4—9.4
	5.07	4.62	5.00	20.34	2.3—8.0
Zinc–histamine–glutaminate	10.15	8.58	10.00	43.89	1.9—7.3
	5.07	8.58	10.00	43.37	1.9—7.8
	5.07	8.58	5.00	32.66	1.9—7.6
	5.07	4.29	10.00	32.66	2.1—7.7
	5.07	4.29	5.00	21.94	2.1—7.5
	10.15	4.29	5.00	22.46	2.1—7.0
Zinc–histamine–threoninate	10.15	8.58	10.00	44.04	2.2—7.0
	5.07	8.58	10.00	43.52	2.3—8.2
	5.07	4.29	10.00	32.77	2.2—7.8
	5.07	8.58	5.00	32.77	2.3—7.8
	5.07	4.29	5.00	22.02	2.4—8.0
	10.15	4.29	5.00	22.54	2.3—6.7
Zinc–histamine–citrate	10.15	8.58	9.97	44.04	2.1—7.8
	5.07	8.58	9.97	43.52	2.1—8.0
	5.07	4.29	9.97	32.77	2.0—8.3
	5.07	8.58	4.99	32.77	2.0—8.0
	5.07	4.29	4.99	22.02	2.2—8.1
	10.15	4.29	4.99	22.54	2.2—6.8

Calculation of Formation Constants.—All of the potentiometric titration data were treated with the MINIQUAD program.[13]

For the binary systems, the composition of the species possibly existing was deduced from the shape of the formation curve of the system. In this case, the average number of ligands bound to each metal ion was obtained

$$\bar{r} = \{C_L - ([L] + [HL] + [H_2L] + \cdots)\}/C_M \quad (2)$$

from equation (2) in which C_L and C_M represent the total metal and total ligand concentrations respectively.

As previously emphasized,[8] the calculation of the experi-

* For details see Notices to Authors No. 7, *J.C.S. Dalton*, 1979, Index issue.

mental function $\bar{r}$ is independent of the metal complex species existing in solution. So, besides the usual examination of the numerical fittings corresponding to the various possible combinations of the formation constants in the same system,[13] the selection of the ' best ' set was finally based on the graphical comparisons made between the experimental function $\bar{r}$ and the theoretical ones, as simulated by the PSEUDOPLOT program.[14] The latter were actually obtained from the free-proton concentrations, iteratively calculated from the set of constants under examination, and the analytically known total reagent concentrations.

TABLE 2

Stability constants $\beta_{pqrs} = [M_rL_pX_qH_s]/[M]^r[L]^p[X]^q[H]^s$ of parent complexes of histamine, cysteinate, histidinate, glutaminate, threoninate, and citrate at 37 °C and $I = 0.15$ mol dm^{-3} Na[ClO$_4$], as used in the related calculations (L = histamine, X = second ligand under consideration)

System	p	q	r	s	logβ	Ref.
Proton–histamine	1	0	0	1	9.426	6
	1	0	0	2	15.315	
Zinc–histamine	1	0	1	0	4.867	6
	2	0	1	0	9.653	
	1	0	1	−1	−2.736	
Proton–cysteinate	0	1	0	1	10.110	8
	0	1	0	2	18.078	
	0	1	0	3	20.050	
Zinc–cysteinate	0	2	1	0	17.905	8
	0	1	1	1	14.604	
	0	2	1	1	24.114	
	0	4	3	0	42.278	
	0	4	3	1	48.313	
	0	4	3	2	54.082	
Proton–histidinate	0	1	0	1	8.770	15
	0	1	0	2	14.643	
	0	1	0	3	16.400	
Zinc–histidinate	0	1	1	0	6.336	15
	0	2	1	0	11.599	
	0	1	1	1	10.718	
	0	2	1	1	16.919	
Proton–glutaminate	0	1	0	1	8.680	9
	0	1	0	2	10.864	
Proton–threoninate	0	1	0	1	8.573	9
	0	1	0	2	10.721	
Proton–citrate (NDP = 3)	0	1	0	1	5.539	8
	0	1	0	2	9.775	
	0	1	0	3	12.644	
Zinc–citrate (NDP = 3)	0	1	1	0	4.715	8
	0	1	1	1	8.441	
	0	2	1	0	7.361	
	0	2	2	−2	−2.214	

As far as the ternary systems are concerned, the same kind of approach was used, except that all the graphical considerations (deductions of the species possibly existing as well as comparisons between experimental and simulated curves) were now based on the average number of protons bound to both of the two ligands under consideration, which was obtained from equation (3).

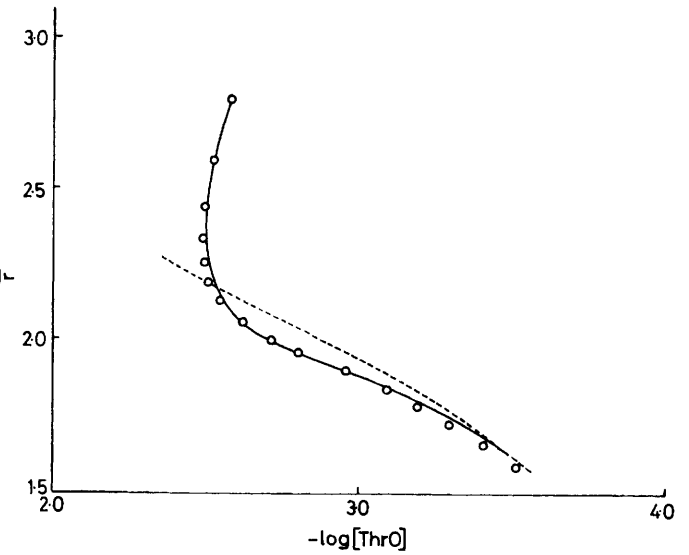


FIGURE 1 Upper part of the zinc–threoninate formation curve. The broken line simulates the existence of $[Zn(ThrO)]^+$, $[Zn(ThrO)_2]$, and $[Zn(ThrO)_3]^-$ (see SUP 22872); the solid-line represents $[Zn(ThrO)]^+$, $[Zn(ThrO)_2]$, and $[Zn(ThrO)_2(OH)]^-$ as given in Table 3; ○ represents experimental points

$$\bar{s} = (C_H + NDP_L \cdot C_L + NDP_X \cdot C_X - C_{OH} + [OH] - [H])/(C_L + C_X) \quad (3)$$

In this equation, C_H, C_L, C_X, and C_{OH} stand for the total concentrations of strong acid, first ligand L, second ligand X, and sodium hydroxide respectively introduced in the solution and NDP for the number of dissociable protons of the related ligands.

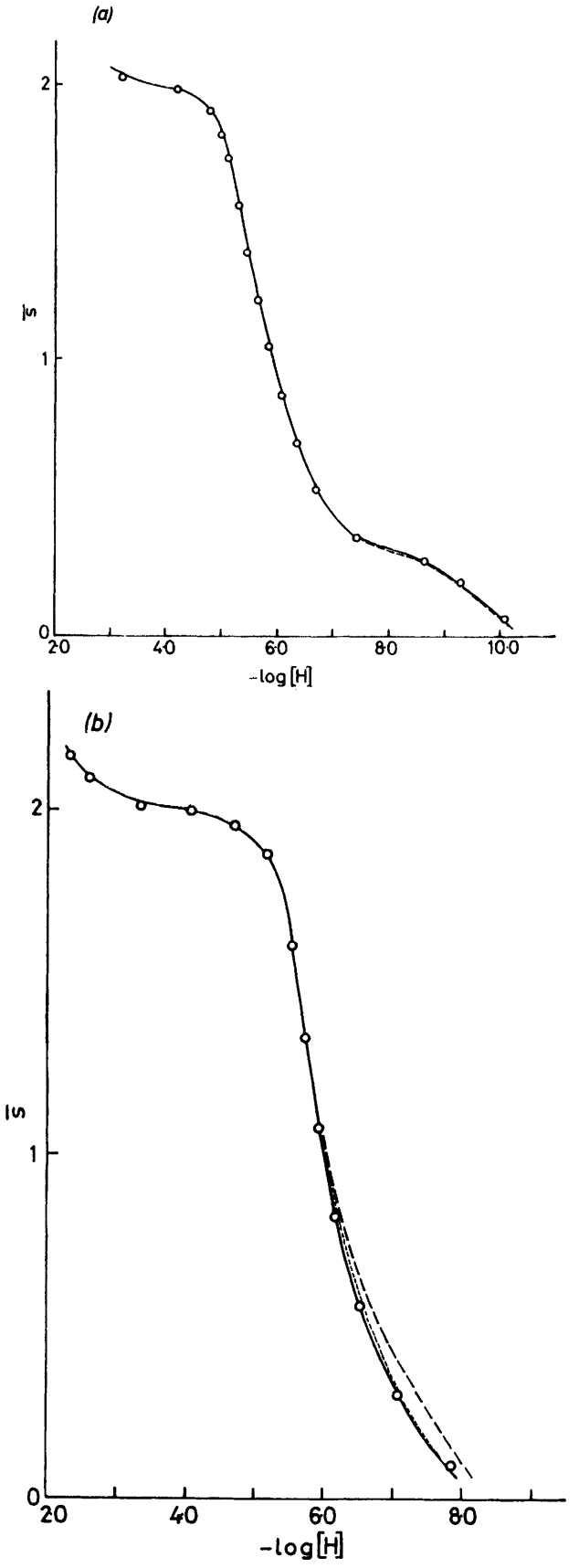
The calculation of the theoretical function $\bar{s}$ corresponding to each set of ternary constants was carried out with a modified version of the PSEUDOPLOT program.[15]

All the parent protonation and binary complex formation constants which were used in the present calculations are shown in Table 2.

TABLE 3

Stability constants obtained from this work. The formula of the general complex is Zn_r(histamine)$_p(X)_q(H)_s$ in which X stands for the second ligand. n = Number of experimental observations, S = sum of squares of residuals

System	p	q	r	s	logβ	S	n
Zinc–histamine–cysteinate	1	1	1	0	14.592 ± 0.023	0.143 × 10^{-5}	300
	1	1	1	1	21.130 ± 0.025		
Zinc–histamine–histidinate	1	1	1	0	11.060 ± 0.009	0.937 × 10^{-6}	300
Zinc–glutaminate	0	1	1	0	4.174 ± 0.007	0.103 × 10^{-5}	142
	0	2	1	0	7.664 ± 0.011		
	0	2	1	−1	−2.137 ± 0.138		
Zinc–histamine–glutaminate	1	1	1	0	9.102 ± 0.025	0.559 × 10^{-5}	300
Zinc–threoninate	0	1	1	0	4.467 ± 0.010	0.337 × 10^{-5}	256
	0	2	1	0	8.279 ± 0.015		
	0	2	1	−1	−1.159 ± 0.044		
Zinc–histamine–threoninate	1	1	1	0	9.311 ± 0.009	0.817 × 10^{-6}	300
Zinc–histamine–citrate	1	1	1	0	9.254 ± 0.057	0.351 × 10^{-5}	300
	1	1	1	1	15.997 ± 0.067		
	1	1	2	−2	−1.222 ± 0.042		



RESULTS

Zinc–Glutaminate System.—The part of the formation curve which was experimentally determined before precipitation occurred in solution exceeded two for the lowest zinc : glutaminate ratio and did not tend towards an obvious limit. As the $[Zn(GlnO)_3]^-$ complex had been previously characterised by Williams,[16] we refined the sets of constants pertaining to, at one time MX, MX_2, and either MX_3 or $MX_2(OH)$, and at another time MX, MX_2, and both MX_3 and $MX_2(OH)$. The best numerical as well as graphical fittings were obtained for the MX, MX_2, and $MX_2(OH)$ species as shown in Table 3. Moreover, the MX_3 constant was made negative by MINIQUAD when refined together with that of $MX_2(OH)$, which confirms the existence of the latter, rather than MX_3, as the minor species under our experimental conditions.

Zinc–Threoninate System.—The experimental observations made for this system are very similar to those related to the previous one. As Sharma [17] had already mentioned the existence of the $[Zn(ThrO)_3]^-$ complex, we tried to refine its equilibrium constant on the basis of our data, but the $[Zn(ThrO)_2(OH)]^-$ species was obtained instead. The PSEUDOPLOT based graphical comparisons led to very clear conclusions in this case, as shown in Figure 1 where the upper part of the formation curve of the system is given.

Zinc–Histamine–Cysteinate System.—It can be seen from Figure 2, which shows two examples of the protonation curves based on equation (3), that ternary complexation is not significant in this system. Indeed, the curves which simulate the experimental data on the basis of binary complexes only are close to the experimental points.

The similar system zinc–histamine–D–penicillamine has already been investigated by Gergely and co-workers,[18] who have characterised the sole species MLX. Nevertheless, for a comparison with the zinc–histidinate–cysteinate system recently studied by one of us,[15] we tested the existence of the two complexes [Zn(histamine)(CysO)] and $[Zn(histamine)(CysO)H]^+$. Both appeared to exist, the second halving the sum of squares when refined together with the first (see SUP 22872). Results are shown in Table 3. Except for the 2nd and 5th experiments in Table 1 in which [Zn(histamine)(CysO)] reached 72% and 50% respectively, neither of these complexes ever represented more than 18% of the metal, which confirms the above expectations.

PSEUDOPLOT simulations based on the corresponding constants accounted for the major part of the experimental curves. However, since zinc can *a priori* bind ligands under various structures and since the species $[Zn(histamine)_3]^{2+}$ had already been mentioned in the literature,[19] we also investigated the possibility of forming species based on the ML_2X and MLX_2 stoicheiometries. Except that of ML_2X, the stability constants of these two complexes as well as those of their protonated derivatives were made negative during MINIQUAD refinements. Moreover, as

FIGURE 2 Protonation curves of mixtures of histamine and cysteine in the presence of zinc: (*a*) $C_{histamine} = 4.62$, $C_{CysO} = 10.01$, $C_{Zn} = 5.07$ mmol dm^{-3}; (*b*) $C_{histamine} = 2.31$, $C_{CysO} = 2.53$, $C_{Zn} = 2.54$ mmol dm^{-3}. The broken line simulates the curves assuming no mixed-ligand species formation; the dotted line is related to the existence of [Zn(histamine)(CysO)] only; the solid line takes account of [Zn(histamine)(CysO)] and $[Zn(histamine)(CysO)H]^+$ as given in Table 3; ○ represents experimental points

ML_2X did not exceed 5% in any of the related experiments and did not improve the sum of squares either (see SUP 22872), it was assumed to be a minor species and so was not included in Table 3.

Zinc–Histamine–Histidinate System.—From the protonation curves shown in Figure 3, one would not expect as weak a ternary complexation as in the previous system.

Following the literature reports mentioning the existence of the species $[Zn(histamine)(HisO)]^+$ [20] and $[Zn(histamine)(HisO)H]^{2+}$,[21] we refined their stability constants, tion in the previous set did not significantly improve the sum of squares and (ii) its maximum percentage was less than 5%. It was thus considered negligible.

Zinc–Histamine–Glutaminate and Zinc–Histamine–Threoninate Systems.—Ternary complexation was not significant in these systems, since the simulated protonation curves, assuming no mixed-ligand complex formation, were almost identical to the experimental ones (see SUP 22872). Moreover, as no protonated complex was found in the parent systems, MLXH was not likely to exist as a ternary species.

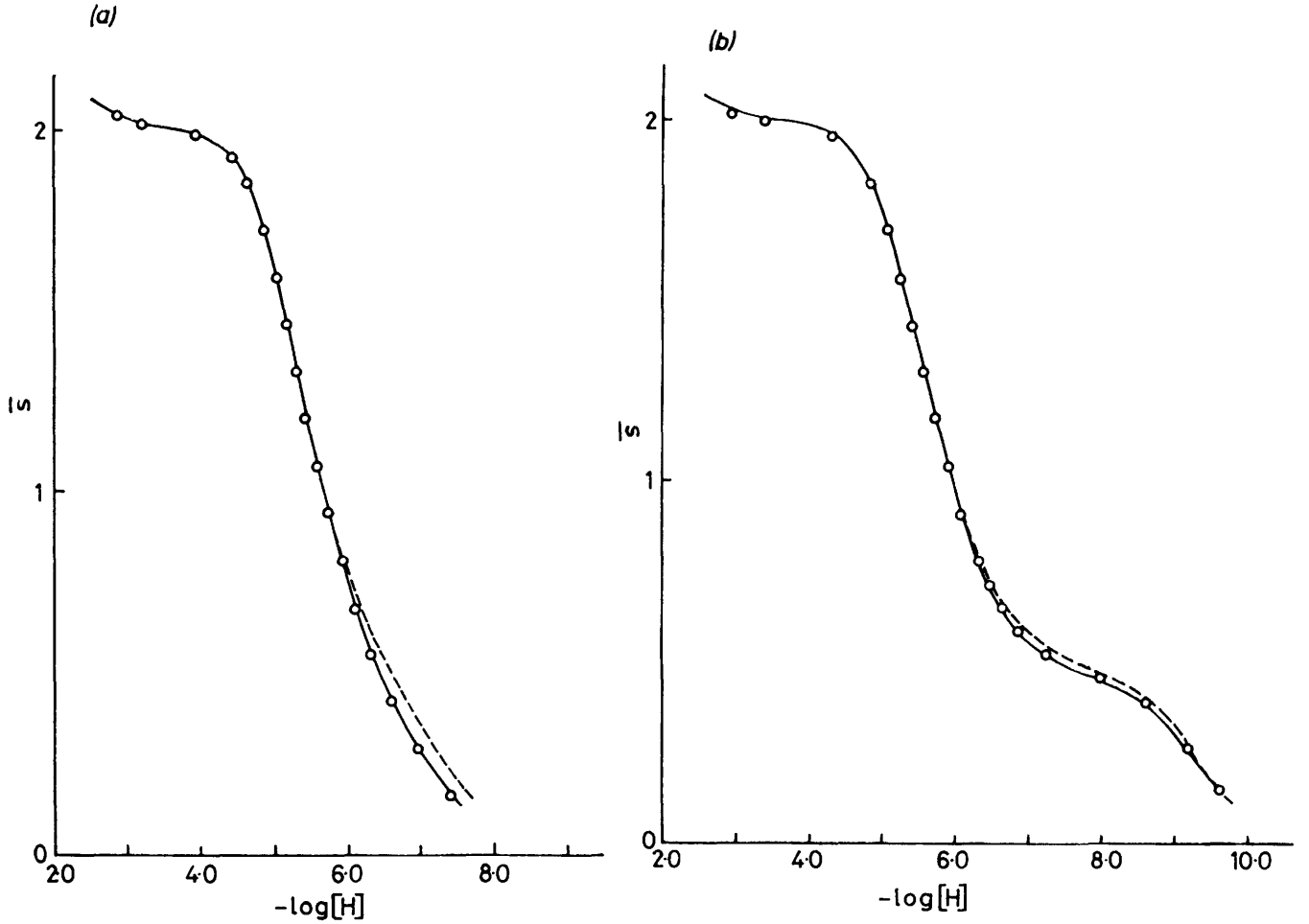


FIGURE 3 Protonation curves of mixtures of histamine and histidine in the presence of zinc: (*a*) $C_{histamine} = 9.23$, $C_{HisO} = 10.00$, $C_{Zn} = 10.15$ mmol dm^{-3}; (*b*) $C_{histamine} = 9.23$, $C_{HisO} = 10.00$, $C_{Zn} = 5.07$ mmol dm^{-3}. The broken line simulates the curves assuming no mixed-ligand species formation; the solid line takes account of $[Zn(histamine)(HisO)]^+$ as given in Table 3; ○ represents experimental points

first each in its turn, then together. We characterised only the $[Zn(histamine)(HisO)]^+$ complex (Table 3), the percentage of which lay between 26% and 51% throughout the experiments, the constant of $[Zn(histamine)(HisO)H]^{2+}$ being made negative during MINIQUAD refinement.

Figure 3 shows that the MLX species alone accounts almost exactly for the differences between the experimental protonation curves and the theoretical ones which were simulated with binary complexes only. Nevertheless, for the above mentioned reason, minor species of the ML_2X and MLX_2 types were also researched. The MLX_2 constant was not made negative (see SUP 22872) but (i) its introduction in the previous set did not significantly improve the sum of squares and (ii) its maximum percentage was less than 5%. It was thus considered negligible.

Accordingly, MLX was the only ternary complex characterised (Table 3), its percentage never exceeding 45% in any system.

Since the species $[Zn(GlnO)_3]^-$ and $[Zn(ThrO)_3]^-$ had already been mentioned in the literature,[16, 17] we envisaged the possible existence of MLX_2 in both systems, but the related constants turned out to be negative in MINIQUAD refinements.

Zinc–Histamine–Citrate System.—This system has already been studied by Daniele and Ostacoli [22] who characterised the $[Zn(histamine)(citrate)]^-$ and $[Zn(histamine)(citrate)H]$ species.

Computational analyses of the present system depend on the choice of NDP for citrate, as for the zinc–citrate system. With NDP = 4, the PSEUDOPLOT approach can be used on the whole range of protonation curves, but the values of the formation constants then vary according to the inaccuracy of the first citrate protonation constant.[8] Thus, as mentioned earlier,[8] the calculations were at first made with NDP = 4 in order to investigate the possibly existing species, then, once these were characterised, the formation constants were refined for NDP = 3 which is considered more satisfactory from the chemical point of view.

The experimental protonation curves fitted almost perfectly with those resulting from the simulation of the binary complexes only (see SUP 22872), so mixed-ligand complexation was expected to be weak. In spite of this observation, the formation constants for the MLX and MLXH complexes already mentioned [22] were not made negative by the MINIQUAD refinements, these species reaching a maximum of 21% and 15% respectively. Nevertheless, it is noteworthy that the sum of squares was almost equivalent whether or not MLX was refined together with MLXH, which would suggest that the existence of the latter could be disregarded.

We also tried to refine the formation constant of $M_2LX(OH)_2$ either in the presence of MLX only, or together with both MLX and MLXH. Although the distribution of $M_2LX(OH)_2$ reached more than 33% in this case, the sum of squares was not significantly improved and the corresponding graphical comparisons did not lead to any clear conclusion (see SUP 22872).

The result of this last set was finally given as the ' best ' in Table 3, but since MLX could be considered as the only species definitely existing, it is worthwhile to note that its stability constant was found to be 9.358 when refined by itself.

TABLE 4

Plasma distribution of histamine as obtained from COMICS simulations *

Species	Electrical charge	Concentration / mol dm^{-3}	Percentage of the complexed histamine fraction
[Zn(histamine)(CysO)]	0	2.09×10^{-13}	75.75
[Zn(histamine)(CysO)H]	+1	2.88×10^{-14}	10.41
[Zn(histamine)(HisO)]	+1	2.18×10^{-14}	7.89
[Cu(histamine)(HisO)]	+1	4.16×10^{-15}	1.50
[Zn(histamine)]	+2	4.13×10^{-15}	1.49
[Zn(histamine)(citrate)]	−1	2.68×10^{-15}	0.97
[Zn(histamine)(OH)]	+1	2.59×10^{-15}	0.94
[Zn(histamine)(GlnO)]	+1	1.84×10^{-15}	0.67
[Zn(histamine)(ThrO)]	+1	1.08×10^{-15}	0.39

* (i) The total ligand concentrations were as follows: $C_{histamine} = 6.2 \times 10^{-9}$ (ref. 24), $C_{CysO} = 2.3 \times 10^{-5}$ (ref. 7), $C_{HisO} = 8.5 \times 10^{-5}$ (ref. 7), $C_{GlnO} = 5.21 \times 10^{-4}$ (ref. 7), $C_{ThrO} = 1.5 \times 10^{-4}$ (ref. 7), $C_{citrate} = 2.70 \times 10^{-5}$ mol dm^{-3} (derived from refs. 7 and 8). (ii) The total metal concentrations were used as: $C_{Zn} \sim [Zn] \neq 10^{-9}$ mol dm^{-3} (the zinc fraction complexed by histamine being negligible with regard to the free zinc concentration in plasma refs. 6—8) and $C_{Cu} = [Cu] + [Cu(histamine)(HisO)]^+ = 4.26 \times 10^{-15}$ mol dm^{-3} {we considered the free concentration of copper to be 10^{-16} mol dm^{-3} as in the latest plasma model used (G. Berthon, C. Matuchansky, and P. M. May, *J. Inorg. Biochem.*, 1980, **13**, 63); the concentration of $[Cu(histamine)(HisO)]^+$ being derived from our previous simulation data (ref. 6) combined with the corresponding formation constant 17.34 lately determined by us (ref. 9)}. (iii) −log [H] was taken as 7.40. (iv) All zinc complex stability constants and protonation constants were used as in Tables 2 and 3.

Distribution of Histamine among Zinc–Histamine–Cysteinate, –Histidinate, –Glutaminate, –Threoninate, and –Citrate Complexes under Plasma Conditions.—An updated version of COMICS [23] was used to simulate the plasma distribution of histamine among the ternary systems investigated in the present study, together with the interfering $[Cu(histamine)(HisO)]^+$ species.[6] This calculation was run on the basis of concentration data derived from our previous simulations,[6] except that this time the total histamine concentration was taken as that found in ref. 24. Results are shown in Table 4.

DISCUSSION

Binary Systems.—For both the zinc–glutaminate and the zinc–threoninate system, most of the previous data available in the literature mentioned only the existence of the two species MX and MX_2. Only Williams [16] and Sharma [17] have found a MX_3 complex in these systems. Our results differ from the latter in that we characterised the species $MX_2(OH)$ instead of MX_3.

No details are given by Sharma [17] concerning the experimental data which led to the calculation of the $[Zn(ThrO)_3]^-$ constant, so it is difficult to interpret the discrepancy between our results, except that a confusion might have arisen between MX_3 and $MX_2(OH)$ in the absence of an unequivocal graphical comparison in his study.

As for the zinc–glutaminate system, Williams [16] found a regular formation curve which clearly tended to three as a maximum limit, but the temperature (25 °C) as well as the ionic strength ($Na[ClO_4]$ 3 mol dm^{-3}) used in this case generally result in larger stability constants,[25,26] favouring the characterisation of complexes of higher degree. Accordingly, it appears that different results are mainly due to the different experimental conditions used.

Ternary Systems.—Generally speaking, zinc can accommodate several binding structures. This kind of competition between different symmetries of complexation for a given metal ion has already been invoked as possibly responsible for the entatic state frequently observed in biological systems.[27] For this reason, various stoicheiometries of zinc mixed-ligand complexes were expected.

However, some limitations may be imposed by factors affecting the parent binary systems. In $[Zn(HisO)_2]$ for instance, the zinc ion is tetrahedrally co-ordinated and the two carboxy-groups are only loosely bound and somewhat distant from the co-ordination centre,[27,28] so that histidinate could be considered as bidentate rather than terdentate in this complex.[28] Thus, on account of the similarity between histidinate and histamine, the octahedral structure seems unlikely in the ternary complexes of zinc with these two ligands. In spite of the already mentioned $[Zn(histamine)_3]^{2+}$ complex,[19] the reasons shown above indicate that the formation of high degree ternary complexes would be severely hindered under the present experimental conditions.

Accordingly, the MINIQUAD refinements of constants of protonated or unprotonated ML_2X and MLX_2 complexes led to negative values or to very poor fits, and we essentially characterised only species of the MLX and MLXH types.

As for the zinc–histamine–cysteinate system, the similarity between the histamine and histidinate ligands resulted in the same complexes as those previously mentioned in the zinc–histidinate–cysteinate system.[15]

For the zinc–histamine–histidinate system, our results confirm those obtained earlier by Gergely and co-workers [20] who characterised the $[Zn(histamine)(HisO)]^+$ species only, but differ from Daniele and Ostacoli's [21] who also calculated the $[Zn(histamine)(HisO)H]^{2+}$ constant. As far as the latter discrepancy is concerned, it could have arisen from the fact that Daniele and Ostacoli did not consider the existence of the $[Zn(HisO)_2H]^+$ species in the parent complexes, which could then be wrongly identified as $[Zn(histamine)(HisO)H]^{2+}$ in ternary constant refinements.

Citrate gave rise to the formation of complexes other than MLX. Once more our results differ from those obtained by Daniele and Ostacoli,[22] in that we suggest the formation of $M_2LX(OH)_2$ as well as MLX and MLXH. This time the authors failed to consider the $[Zn(citrate)_2]^{4-}$ complex among the parent binary species, which could explain the difference observed.

Let us now consider the ability of zinc and histamine to form ternary complexes with the ligands investigated here. This tendency may be appreciated by examining the specific increments of stability which account for the formation of mixed-ligand species relative to the corresponding parent ones.

The calculation of these increments is possible using two basic equations [(4) and (5)].[29]

$$\Delta \log K = \log K^{ML}_{MLX} - \log K^{M}_{MX} = \log K^{MX}_{MLX} - \log K^{M}_{ML} = \log \beta_{1110} - \log \beta_{1010} - \log \beta_{0110} \quad (4)$$

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

Equation (4) expresses the ability of a ML or MX complex to bind a second ligand X or L rather than L or X respectively, whereas equation (5) accounts for the tendency of a ternary system to give rise to a MLX complex, more, or less, stable than expected on purely statistical grounds. Table 5 gives values, thus derived, for the systems investigated, as well as those derived from literature data for comparison.

The following observations may be made. (i) The most favoured ternary complex characterised in this study is [Zn(histamine)(CysO)], which appears to be more stable than [Zn(histamine)(D-penicillaminate)] [18] and far more than $[Zn(hisO)(CysO)]^-$.[15] This 'over-stabilisation,' which should be of some importance to the plasma distribution of histamine (to be discussed later), shows that histamine is more prone than histidinate to form ternary complexes with cysteine. (ii) The formation of $[Zn(histamine)(HisO)]^+$ is less favoured than that of [Zn(histamine)(CysO)], presumably because of the similarity of the two ligands as regards the nature of their donor atoms.[30] The fact that β^{M}_{MX} is markedly lower than $\beta^{MX}_{MX_2}$ for cysteine (see data deposition in ref. 8) but not for histidine [15] could also play a part in this phenomenon.[20] (iii) The mixed-ligand complex stabilisation for glutaminate and threoninate is greater than for glycinate. This could arise from the possible involvement of the amide and hydroxy-groups in the ternary complexation respectively, as is indicated by the value already observed [19] for the $[Zn(histamine)(SerO)]^+$ (SerO = serinate) species. (iv) As already pointed out by Daniele and Ostacoli,[22] histamine forms more stable mixed-ligand complexes with citrate than histidinate does. The absence of oxygen as a possible donor atom in histamine could partly explain this.[30] It is also noteworthy that this observation is in line with the above remark concerning the different behaviour of histamine and histidinate towards cysteinate in the zinc ternary complexation.

Simulated Distribution of the Main Histamine Complexes under Plasma Conditions.—The COMICS simulation shown in Table 4 confirms that, as already sug-

TABLE 5

Increments of stability for the formation of ternary complexes of zinc and histamine with various amino-acids and citrate, and for the zinc–histidinate–cysteinate and zinc–histidinate–citrate systems given for the sake of comparison

System	$\Delta \log K$	$\Delta \log \beta$	Ref.	Experimental conditions
Zinc–histamine–cysteinate	1.12	0.51	This work	
Zinc–histamine–D-penicillamine	0.85	0.13	18	25 °C, KCl 0.2 mol dm^{-3}
Zinc–histamine–histidinate	−0.14	0.13	This work	
	−0.39	0.11	20	25 °C, KCl 0.2 mol dm^{-3}
	−0.21	0.21	21	25 °C, $K[NO_3]$ 0.1 mol dm^{-3}
Zinc–histamine–glutaminate	0.06	0.14	This work	
Zinc–histamine–threoninate	0.00	0.06	This work	
Zinc–histamine–citrate	−0.33	0.45	This work	
	−0.38		22	25 °C, $K[NO_3]$ 0.1 mol dm^{-3}
Zinc–histamine–glycinate	−0.29	−0.07	*a*	25 °C, $K[NO_3]$ 0.1 mol dm^{-3}
Zinc–histamine–serinate	0.17	0.31	19	37 °C, $K[NO_3]$ 0.15 mol dm^{-3}
Zinc–histidinate–cysteinate	0.15	0.04	15	37 °C, $Na[ClO_4]$ 0.15 mol dm^{-3}
Zinc–histidinate–citrate	−1.15	−0.12	*b*	37 °C, $Na[ClO_4]$ 0.15 mol dm^{-3}
	−0.99		22	25 °C, $K[NO_3]$ 0.1 mol dm^{-3}

[a] P. G. Daniele and G. Ostacoli, *J. Inorg. Nuclear Chem.*, 1978, **40**, 1273. [b] M. J. Blais and G. Berthon, unpublished work.

gested,[6] [Zn(histamine)(CysO)] is by far the most concentrated complex of histamine in normal blood plasma.

The present distribution is limited to the main histamine complexes as previously predicted on statistical grounds [6] so its order could be slightly modified in the future by the introduction in the simulation model of experimental constants pertaining to complexes hitherto considered as less important species. Nevertheless, it now seems most likely that none of these minor species could reach the level of [Zn(histamine)(CysO)].

This observation suggests that the zinc-induced decrease of the pharmacological activity of histamine [5] might arise from the electroneutrality of the predominant [Zn(histamine)(CysO)] complex, which allows histamine to diffuse into tissues, where it is immediately catabolised.[31] Conversely, the effect of copper which is known to aggravate the histamine toxicity [5] might be due to the formation of the positively charged [Cu(histamine)(HisO)]$^+$ complex, for the predominance of this species would tend to prolong the lifetime of histamine in the blood stream.

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