Metal Ion–Tetracycline Interactions in Biological Fluids. Part 4. Potential Influence of Ca^{2+} and Mg^{2+} Ions on the Bioavailability of Chlortetracycline and Demethylchlortetracycline, as Expected from their Computer-Simulated Distributions in Blood Plasma

LUC LAMBS, MICHEL BRION and GUY BERTHON*

Centre de Technologie Biomédicale INSERM SC 13, Laboratoire de Chimie Bioinorganique, Université Paul Sabatier, 38, rue des Trente-Six Ponts, 31400 Toulouse, France

Received September 17, 1984

Abstract

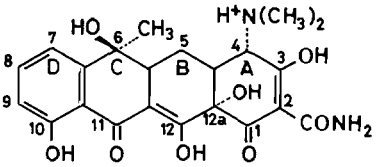
Coordination of tetracyclines with calcium and magnesium was previously shown to exert a determining effect on the distribution of these antibiotics in blood plasma. In particular, it was clearly established by computer simulation that the free fraction of the drug is quite negligible with respect to its metal-bound fraction. The bioavailability of a tetracycline in blood plasma is thus expected to depend directly on the electrical charge of its predominant metal complexes in the biofluid. On account of the metal to ligand ratio corresponding to the usual therapeutic levels, bioavailability is critically sensitive to the property of the antibiotic to give rise to electrically charged binuclear species. The blocking of one of the two potential binding sites of the tetracycline molecule should thus result in a larger percentage of neutral complexes, hence in a better tissue penetration by the drug.

The present work is devoted to the investigation of the coordination of 7-chlortetracycline (CTC) and 6-demethyl-7-chlortetracycline (DMC) with calcium and magnesium in blood plasma. The influence of the chloro substituent is discussed with respect to the objective defined above.

Introduction

In the recent past, much attention has been paid to the characterization of the different conformations of the tetracycline molecule [1–4], particularly with a view to distinguishing the form likely to allow passive diffusion of the antibiotic through cell membranes [5–7]. Indeed, it has long been considered that the distribution of tetracyclines in blood plasma involves two distinct fractions: (i) a protein-bound pool which acts as a storage reserve and (ii) a free fraction which is considered to be the available form of the drug which will eventually reach its therapeutic target [8].

Various metal ions do occur naturally in biofluids [9] and it has been suggested that some of them might well interfere with the bioavailability of tetracyclines [8, 10, 11], but, due to a lack of reliable quantitative data, their influence has been widely neglected. It was not until our preliminary study of the calcium interactions with four tetracyclines (namely tetracycline: TC (I), oxytetracycline: OTC,



I

doxycycline: DOXY and minocycline: MINO) that it was clearly established by computer simulation that the so-called free fraction [8] of tetracyclines in blood plasma was actually complexed with Ca(II) to a considerable extent [12]. As a matter of fact, taking into account the influence of the sole Ca(II) ions resulted in a percentage of really free antibiotic situated well below 1% [12].

As the concentration of magnesium in blood plasma is almost of the same order of magnitude as that of calcium, the complex equilibria of this metal with the same four tetracyclines were subsequently investigated. The outcome of this study confirmed the insignificance of the free fraction of these tetracyclines in blood plasma at therapeutical levels [13]. Moreover, the characterisation of binuclear complexes of magnesium as well as calcium raised the possibility that complexes including both of these metals are formed. This hypothesis proved to be true for TC, OTC and MINO, and the corres-

*Author to whom correspondence should be addressed.

0020-1693/85/$3.30

ponding computer simulations recently reported [14] show the relative importance of these species under *in vivo* conditions.

All these results revealed that the physico-chemical properties of the free tetracycline molecules, such as their conformations [4–7] or their partition coefficients [8, 15, 16], should not be considered as the determining criteria to assess the bioavailability of these antibiotics in blood plasma. Instead, the most critical property is the electrical charge, hence the stoichiometry of the predominant calcium and/or magnesium complexes that these substances are likely to form in the biofluid.

Plasma concentrations of calcium and magnesium being several orders of magnitude higher than the usual therapeutic levels of tetracyclines, the bioavailability of an antibiotic of this class clearly depends on its formal ability (or rather inability) to form electrically charged homo- or heterobinuclear complexes with these two metals. It would thus seem logical that blocking one of the two potential binding sites of a tetracycline molecule would tend to favour its overall bioavailability. The C_{10}, C_{11}, C_{12} site is known to be involved in the coordination of calcium [1, 3, 4, 11, 17, 18] as well as magnesium [3, 11, 18] in aqueous solution, and its electron density may be weakened by the substitution of an electro-attractor group in C_7. The present papers thus reports the results of a potentiometric investigation of the complex equilibria of 7-chlorotetracycline (CTC) and 6-demethyl-7-chlortetracycline (DMC) with calcium and magnesium. The possible effects of the chloro substituent with respect to the bioavailability of these tetracyclines in blood plasma are discussed on the basis of the relevant computer-simulated distributions.

Experimental

Formation Constant Determinations

Reagents

Standard stock solutions of calcium and magnesium were prepared as their chloride salts using BDH Analar grade and Prolabo R.P. Normapur grade crystals respectively, in accordance with the specifications given earlier [12–14].

DMC was kindly supplied as free base by Lederle Lab. whereas CTC hydrochloride was purchased from Sigma Chemical Co. On account of the well documented instability of tetracyclines in aqueous media [1, 19], especially CTC [3], their solutions were frequently renewed and systematically analysed before use for their antibiotic and acid contents, by means of the appropriate potentiometric Gran titrations [20]. The solutions as well as the original products were stored in the absence of light under an atmosphere of purified nitrogen.

The sodium chloride used as a background electrolyte was obtained from Merck as a pro analysi product. The titrant sodium hydroxide solutions containing sodium chloride 0.15 mol dm^{-3} were prepared under a nitrogen blanket, by diluting BDH concentrated volumetric solutions in freshly boiled doubly deionised water, and they were standardized as described previously [12]. The stock solution of hydrochloric acid was also prepared from a BDH concentrated solution and its titre was potentiometrically checked.

Equipment

The titration unit, involving a Beckman Model 4500 digital mV-meter, an Ingold cell system and a Metrohm 645 Multidosimate burette, was basically the same as that used in our previous studies in this series [12–14].

The electrochemical cell was of the following type:

Glass electrode/Antibiotic, Ca^{+2} and/or Mg^{+2},

$$\text{NaCl (0.15 mol dm}^{-3}\text{)/NaCl/Hg}_2\text{Cl}_2\text{–Hg (satd)} \quad (1)$$

using Beckman glass and saturated sodium chloride calomel electrodes.

Experimental Conditions

All the titrations were carried out under a constant bubbling of nitrogen. The temperature was maintained at 37 °C and the ionic background was NaCl 0.15 mol dm^{-3}, this being isotonic with blood plasma.

For the tetracycline protonation studies, the initial solutions of antibiotic contained a sufficient amount of hydrochloric acid for all the coordination sites to be protonated before the outset of the experiment. For the calcium and magnesium complexation studies, one of the metals (for binary systems) or both of them (for ternary systems) were also incorporated in these solutions. As is common for tetracyclines [12–14], a slight opacity often appeared in the metal-containing solutions during the course of the titrations, sometimes from the very beginning of the complexation process. The experiments were nevertheless pursued as long as stable e.m.f. values could be obtained, *i.e.* until a steady drift was observed in the mV-meter readings.

The various reagent concentrations used for the study of each system are reported in Table I, together with the corresponding pH* ranges investigated.

*pH is given for $-\log[H]$ throughout this study, the electrode system being calibrated in terms of proton concentrations.

TABLE I. Summary of the Titration Data Used for Calculating Stability Constants. The initial total concentrations of antibiotic (C_L), calcium (C_{Ca}), magnesium (C_{Mg}) and mineral acid (C_H) in the titrate as well as the hydroxide concentration (C_{OH}) in the titrant are expressed in mmol dm^{-3}; pH stands for $-\log [H]$ (see text).

System	C_L	C_{Ca}	C_{Mg}	C_H	C_{OH}	pH-range
H^+–CTC	1.04			1.94	10.1	2.87–10.08
	1.04			2.90	10.1	2.65– 9.80
	2.07			3.89	10.1	2.58–10.15
Ca^{2+}–CTC	2.02	0.20		3.87	10.1	2.62– 7.98
	2.02	0.33		3.89	10.1	2.56– 7.36
	2.02	0.50		3.90	10.1	2.56– 6.74
	2.02	1.00		3.96	10.1	2.60– 6.29
	1.01	0.50		1.98	10.1	2.84– 6.78
	1.01	1.00		2.03	10.1	2.82– 6.89
	1.01	2.00		2.14	10.1	2.80– 6.73
	0.50	1.00		1.07	10.1	3.05– 6.61
Mg^{2+}–CTC	2.07		0.20	3.91	10.1	2.57– 7.70
	2.07		0.33	3.93	10.1	2.61– 7.06
	2.07		0.50	3.95	10.1	2.59– 6.85
	2.07		1.00	4.01	10.1	2.58– 6.72
	1.04		1.00	2.06	10.1	2.82– 6.65
	1.04		2.00	2.18	10.1	2.83– 6.80
	1.03		1.00	2.02	10.1	2.84– 7.10
	1.03		2.00	2.14	10.1	2.81– 6.85
	0.52		1.00	1.07	10.1	3.10– 7.45
	0.52		1.00	1.07	10.1	3.07– 7.51
Ca^{2+}–Mg^{2+}–CTC	1.00	1.03	1.00	2.13	10.1	2.78– 6.65
	0.50	0.52	0.50	1.07	10.1	3.02– 7.07
	2.00	2.06	2.00	4.26	10.1	2.50– 6.19
	1.00	1.03	1.00	2.13	10.1	2.77– 6.73
H^+–DMC	1.00			1.98	10.1	2.85–10.12
	1.49			2.96	10.1	2.70–10.05
	1.99			3.95	10.1	2.58–10.15
Ca^{2+}–DMC	1.99	0.20		3.97	10.1	2.58– 9.37
	2.49	0.40		4.98	10.1	2.46– 8.91
	2.49	0.60		5.00	10.1	2.45– 8.55
	2.49	1.25		5.07	10.1	2.45– 6.51
	2.49	2.50		5.21	10.1	2.45– 6.18
	2.49	5.01		5.48	10.1	2.41– 6.10
	1.25	2.50		2.74	10.1	2.69– 6.38
Mg^{2+}–DMC	1.88		0.20	3.94	10.0	2.59– 9.67
	2.34		0.40	4.95	10.0	2.51– 7.37
	2.34		0.60	4.98	10.0	2.52– 7.47
	2.34		1.25	5.06	10.0	2.48– 6.61
	2.34		2.50	5.21	10.0	2.48– 6.28
	2.34		5.01	5.51	10.0	2.41– 6.09
	1.17		2.50	2.75	10.0	2.71– 6.56
Ca^{2+}–Mg^{2+}–DMC	1.99	2.00	2.00	4.41	10.1	2.52– 6.05
	1.00	1.00	1.00	2.20	10.1	2.79– 6.69
	0.50	0.50	0.50	1.10	10.1	3.07– 6.95

TABLE II. Stability Constants of the Complexes of Calcium and Magnesium with CTC and DMC at 37 °C in Aqueous Medium NaCl 0.15 mol dm^{-3}. The general formula of a complex is $M_qL_pH_r$; S = sum of squared residuals; R = R factor (see ref. [21]); n = number of experimental observations.

System	$p\ q\ r$	log β	±	S	R	n
H^+–CTC	1 0 1	8.698	0.005	0.167E – 7	0.00441	131
	1 0 2	15.714	0.009			
	1 0 3	18.794	0.012			
Ca^{2+}–CTC	2 1 2	25.230	0.026	0.105E – 7	0.00368	136
	1 1 2	17.984	0.088			
Mg^{2+}–CTC	2 1 2	23.995	0.051	0.171E – 7	0.00546	79
	1 1 1	11.515	0.108			
Ca^{2+}–Mg^{2+}–CTC	No complex in evidence					108
H^+–DMC	1 0 1	8.695	0.004	0.156E – 7	0.00444	125
	1 0 2	15.338	0.008			
	1 0 3	18.474	0.012			
Ca^{2+}–DMC	2 1 2	24.269	0.015	0.344E – 7	0.00434	192
	2 1 1	16.355	0.061			
	1 1 0	4.569	0.051			
	1 2 0	8.343	0.017			
Mg^{2+}–DMC	2 1 2	23.592	0.018	0.269E – 7	0.00354	233
	2 1 1	15.416	0.110			
	1 1 1	11.852	0.012			
	1 2 0	7.605	0.038			
Ca^{2+}–Mg^{2+}–DMC	No complex in evidence					68

Calculation Procedures

For each system investigated, the discrimination of the 'best' set of complexes likely to account for the experimental data and the refinement of the corresponding stability constants were carried out simultaneously by means of our usual two-stage approach [12–14].

The calculations specific to the two successive optimisation/simulation steps were performed by means of the MINIQUAD [21] and PSEUDOPLOT [22] programmes. The details of the procedures actually used have been thoroughly described elsewhere [23, 24], hence no further development need be presented here. It is only noteworthy that the graphical comparisons between experimental and PSEUDOPLOT-simulated data were based on metal–ligand formation curves for binary systems, and on protonation curves for mixed-metal complexation studies as well as for proton–ligand equilibria.

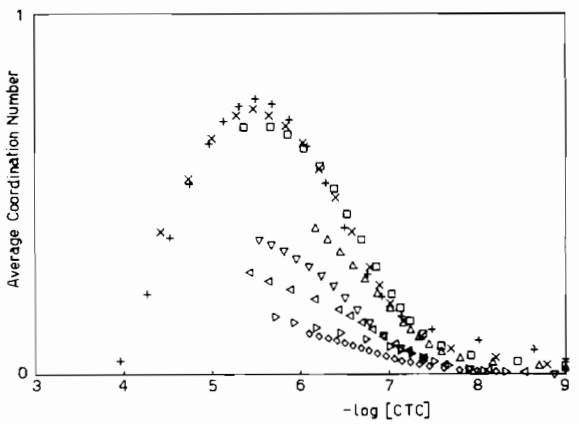


Fig. 1. Experimental formation curve of the calcium–CTC system. The following symbols correspond to the respective order of the experiments summarized in Table I: +, ×, □, △, ▽, ◁, ▷, ◇.

Results and Discussion

The formation constants obtained from these studies are to be found in Table II. Details on the investigation of the corresponding systems are reported elsewhere [25]. Therefore, only the experimental and simulated formation curves of the calcium–CTC system, respectively shown in Figs. 1 and 2, are given as examples illustrating the above-mentioned approach.

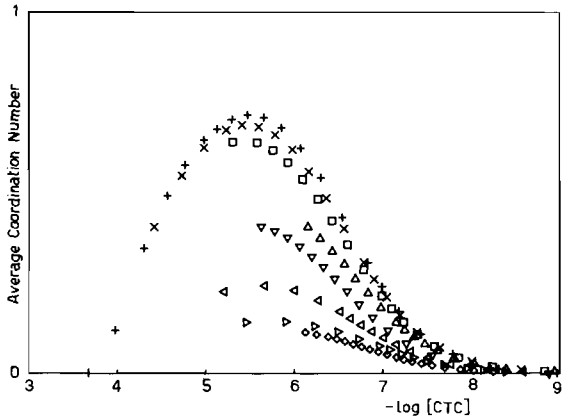


Fig. 2. Simulated formation curve of the calcium–CTC system as obtained from the PSEUDOPLOT programme on the basis of the results shown in Table II. The symbols are the same as in Fig. 1.

The first remark to be made about these results concerns the protonation constants of the two tetracyclines. It was pointed out in the introduction that a certain weakening of the electron density around the C_{10}–C_{11} positions could be expected from the substitution of the chloro group in C_7. According to Leeson *et al.* [26], the first dissociation constant of a tetracycline is associated with the tricarbonylmethane system, the second with the phenolic diketone moiety, and the third with the dimethyl-ammonium ion. It appears from the comparison of the protonation constants in Table II with those of the parent tetracycline [12] that the proton ionisation of the tricarbonylmethane system of CTC and DMC is not affected by the presence of the chloro substituent. However, the constants associated to the other two sites are found to be lower for CTC than for TC (–0.26 and –0.35 respectively), which confirms earlier observations [26, 27]. As for DMC, the effect observed is even more important for the dissociation of the phenolic diketone group (–0.41), but equal for that of the dimethylammonium ion (–0.36).

In the calcium–CTC system, the major complex is undoubtedly ML_2H_2, all the other species refined together with it being negligible or eliminated by MINIQUAD [25], except MLH_2. In a first approach, it may be inferred from the ionisation sequence characterized by Leeson *et al.* [26] that the calcium involved in the MLH_2 complex binds CTC to its tricarbonylmethane system. However, the first calcium ion coordinated to a tetracycline is generally thought to be bound to the $O_{10}O_{11}$ atoms of the diketone moiety [1, 3, 11, 17, 18], this being substantiated by the fact that isochlorotetracycline, which contains only the dimethylamino-group and the C_1–C_2–C_3 system as potential binding sites, does not chelate calcium [28]. For favorable metal to ligand concentration ratios, a second calcium ion may then be bound at higher pH, through the A ring between the 4-NMe_2 and 12_a-OH donor groups [1, 3, 17]. Alternatively, it has also been contended that one metal ion may coordinate with both the BCD and A rings [4], the principal binding site being expected to utilize ligand atoms O_1, O_{12} and O_{11}; a second cation may then coordinate with the N atom of the dimethylamino group [4]. The O_{12}, O_1 site has also been suggested to be involved in the formation of the binuclear complex of calcium with tetracycline at pH 7.4 [11]. It results from these considerations that the calcium ion bound to CTC in the MLH_2 complex would rather coordinate through the O_{12}, O_1 potential donor atoms. This implies that the proton still present on the phenolic diketone moiety in the MLH_2 species (first complex of this type to be characterized between calcium and a tetracycline) would be localized on the O_{10}, O_{11} atoms, this implying in turn that the competition between the calcium and hydrogen ions for this site would turn in favour of the latter. As far as the ML_2H_2 species is concerned, presumably only the dimethylamino group of the ligand is protonated. Quite logically, no binuclear complex could be found in this system, even when specifically researched in the most favorable 2:1 metal to ligand concentration ratio.

The CTC affinity was observed to be poorer for magnesium than for calcium, especially in the low pH range. Among the 246 experimental points originally collected in the first six experiments summarized in Table I, only 79 were actually relevant to any significant complexation and were thus used for the calculations whose results are shown in Table II. No binuclear complex could be found under these conditions. In accordance with our former strategy [12, 13], the potential binuclear species were then researched in the 2:1 metal to ligand ratio experiment. Moreover, on account of the small number of points in the latter, four additional experiments were performed with a specific view to researching these species, but unsuccessfully. MLH and ML_2H_2 were thus recognized as the two major complexes in this system. After Mitscher *et al.* [3], a first magnesium ion can bind tetracycline at the BCD juncture, but the question of the location of the potential second one is still unsolved. Newman and Frank [11] assigned the O_{11}, O_{12} site to the 1:1 complex formed at pH 7.4, which is in line with the above. In contrast, Jogun and Stezowski [4] contended that the first magnesium ion, like calcium, might be expected to coordinate through the O_1, O_{12}, O_{11} atoms, whereas the second would rather bind to the nitrogen of the dimethylamino group. More recently, Martin [18] stated that it was difficult to distinguish between the C_{10}, C_{11}, C_{12} and C_1, C_2, C_3 sites, but that the C_4 nitrogen was not involved in the coordination. It is impossible to draw any

TABLE III. Simulated Distribution of 7-Chlortetracycline (CTC) and 6-Demethyl-7-chlortetracycline (DMC) in their Ten Most Concentrated Complexes in Blood Plasma During Treatment. The total concentration of antibiotic is considered to be 1.0×10^{-5} mol dm^{-3}. The free metal concentrations are taken from ref. [31]. pH is fixed at 7.40.

Antibiotic	Species composition[a]	log β	Percentage	Electrical charge
CTC	CTC–H	8.70	31.6	−1
Free concentration	Ca–$(CTC)_2$–$(H)_2$	25.23	15.4	0
= 1.590×10^{-7} mol dm^{-3}	CTC–$(H)_2$	15.71	13.0	0
(1.59%)	Mg–CTC–H	11.52	10.8	+1
	Ca–CTC–LTA–H	13.91	10.3	0
	Ca–CTC–PO4–$(H)_2$	25.57	3.5	−1
	Ca–CTC–$(H)_2$	17.98	2.7	+2
	Ca–CTC–CTA–H	14.91	1.6	−2
	Ca–CTC–GLN–$(H)_2$	22.12	1.0	+1
	Mg–CTC–LTA–H	13.20	0.9	0
DMC	$(Ca)_2$–DMC	8.34	74.3	+2
Free concentration	Ca–DMC	4.57	11.1	0
= 2.640×10^{-8} mol dm^{-3}	DMC–H	8.69	5.2	−1
(0.26%)	Mg–DMC–H	11.85	3.9	+1
	$(Mg)_2$–DMC	7.60	2.9	+2
	DMC–$(H)_2$	15.34	0.9	0
	Ca–DMC–LTA–H	13.43	0.6	0
	Ca–DMC–PO4–$(H)_2$	25.08	0.2	−1
	Mg–DMC–LTA–H	13.00	0.1	0
	Ca–DMC–CTA–H	14.43	0.1	−2

[a]LTA = lactate, PO4 = phosphate, CTA = citrate, GLN = glutaminate.

clearcut conclusion from our results, which are not aimed at providing any deep insight into the bonding modes. Nevertheless, it is most likely that the dimethylamino group of CTC is protonated in the two complexes mentioned in Table II. It would thus seem logical that the magnesium ion may be linked near the C_{11}, C_{12}, C_1 site, the more so as the presence of the chloro substituent is expected to weaken the electron density in the vicinity of the O_{10} atom.

In view of the absence of binuclear species in both of the binary systems, no mixed-metal complex was expected to be formed between calcium, magnesium and CTC. This was experimentally confirmed (Table II).

The results concerning the coordination of DMC with the two metals are more similar to those already observed for the other tetracyclines previously investigated [12, 13]. For example, DMC forms the same magnesium complexes as doxycycline and shares the same predominant calcium and magnesium complexes with the other derivatives [12, 13]. In spite of the variation of the protonation constants associated with its phenolic diketone and dimethylamino groups (Table II), it thus seems that the structural modifications of the DMC molecule do not significantly affect its mode of coordination with the two above metals. The only noticeable effect is the decrease of stability observed for all of its complexes.

The existence of M_2L binuclear complexes of DMC with calcium and magnesium may lead to the formation of the corresponding mixed-metal species. Surprisingly, the constant of the latter was made negative during MINIQUAD refinement. This suggests that the coordination site of the second metal ion in the binuclear species is different for calcium and magnesium, and specific to the first metal bound.

Computer Simulation Studies

It may be worth recalling that the ultimate objective of the present study was to assess the influence of the C_7-substituted chloro group on the potential capacity of CTC and DMC to diffuse from blood plasma into tissues. As expected from the principles developed in the introduction, this capacity will essentially depend on the electrical charge of the

complexes of the drug that prevail in plasma. Therefore, such an analysis requires a prior knowledge of the distribution of each antibiotic in the various species it gives rise to in the biofluid.

Simulated Distribution of CTC and DMC in Blood Plasma

The simulated distribution of CTC and DMC in blood plasma was obtained by means of the ECCLES programme [29] after incorporating the formation constants reported in Table II into the relevant model [29–32]. Free calcium and magnesium concentrations were respectively taken as 1.13×10^{-3} mol dm^{-3} and 5.2×10^{-4} mol dm^{-3} [29]. The overall concentration of CTC as well as of DMC was considered to be 1.0×10^{-5} mol dm^{-3}, which is of the order of magnitude of the usual plasma level of this kind of antibiotic during treatment [12]. This concentration does not take the plasma protein interactions into consideration, but this is insignificant in the present case since taking these interactions into account would result in an even larger excess of metal with respect to the tetracyclines [13, 14].

Table III shows the distribution of CTC and DMC in their ten most concentrated complexes in blood plasma, as simulated on the basis of the data described above. It is noteworthy that the formation constants of the ternary complexes involving ligands occurring naturally in plasma are statistically generated by the general formula

$$\log \beta_{MLXH_r} = \tfrac{1}{2}(\log \beta_{ML_2H_{r'}} + \log \beta_{MX_2H_{r''}}) + \log 2 \quad (2)$$

The corresponding free concentrations are also mentioned.

Discussion

The first point to be noted is that metal coordination occurs with CTC to a much lesser extent than with DMC and the other tetracyclines investigated in our previous studies [14]. Contrary to what was observed for all the other antibiotics of the series, the proton-associated species are predominant in the low-molecular-weight fraction of CTC in blood plasma, the monoprotonated form being the most important complex of the distribution. In this respect, it is worth recalling that the percentage of the main protonated form of the other tetracyclines was never superior to 5% at the most [14]. The low extent of metal chelation also explains why the free concentration of CTC is found to be much higher than that usually observed.

The direct consequence of the above is the relative importance of the ternary mixed-ligand species of calcium and, to a lesser degree, magnesium in the CTC distribution. In particular, the percentage of the protonated mixed-ligand complex of calcium with lactate and CTC amounts to more than 10%. For the other tetracyclines, this complex also represents the main ternary species given rise to by the ligands occurring naturally in plasma, but its percentage is always inferior to 1. The latter observation is of particular interest since this species is electrically neutral.

TABLE IV. Percentage of Each Antibiotic Present in the Form of Neutral Complex at Therapeutic Level in Blood Plasma.

Antibiotic	Percentage of neutral form
TC	4.7%
OTC	21.4%
DOXY	20.8%
MINO	16.3%
CTC	40.4%
DMC	12.7%

Table IV reports the percentage of each tetracycline present in the form of neutral complexes in plasma, as deduced from our latest simulations under the conditions defined in Table III. The percentages concerning TC, OTC, DOXY and MINO may differ slightly from those observed in our earlier work [14]. This stems from the fact that the influence of other plasma ligands likely to form ternary complexes with tetracyclines and calcium or magnesium had been previously neglected (see above).

As pointed out in the introduction, the ability of a given tetracycline to diffuse through cell membranes is expected to depend on the percentage of the neutral species that it forms in blood plasma. The tissue penetrating abilities of the six antibiotics investigated should thus be in the order:

$$CTC \gg OTC \sim DOXY > MINO > DMC > TC$$

The stability constant of the mixed-ligand complex of calcium and lactate with CTC has been estimated on statistical grounds, which may affect the reliability of the percentage calculated for CTC. Nevertheless, it is obvious from Table III that the above order will remain unchanged irrespective of the constant. The only change likely to affect the above classification would arise from the different physico-chemical properties of the complexes, which closely depend on those of the pertaining ligands.

To conclude, the influence exerted by the chloro substituent on the coordination of tetracyclines to calcium and magnesium is observed to be determining for CTC, but not significant for DMC.

References

1 L. A. Mitscher, A. C. Bonacci and T. D. Sokoloski, *Antimicrob. Agents Chemother.*, 78 (1968).
2 L. A. Mitscher, A. C. Bonacci and T. D. Sokoloski, *Tetrahedron Lett., 51,* 5365 (1968).
3 L. A. Mitscher, B. Slater-Eng and T. D. Sokoloski, *Antimicrob. Agents Chemother., 2,* 66 (1972).
4 K. H. Jogun and J. J. Stezowski, *J. Am. Chem. Soc., 98,* 6018 (1976).
5 J. J. Stezowski, *J. Am. Chem. Soc., 98,* 6012 (1976).
6 L. J. Hugues, J. J. Stezowski and R. E. Hughes, *J. Am. Chem. Soc., 101,* 7655 (1979).
7 G. W. Everett Jr., J. Gulbis and J. Shaw, *J. Am. Chem. Soc., 104,* 445 (1982).
8 M. Schach von Wittenau and R. Yeary, *J. Pharmacol. Exp. Ther., 140,* 258 (1963).
9 D. R. Williams, 'The Metals of Life', Van Nostrand Reinhold, London, 1971.
10 A. Albert, in 'Strategy in Chemotherapy', Cambridge University Press, London, 1958, p. 112.
11 E. C. Newman and C. W. Frank, *J. Pharm. Sci., 65,* 1728 (1976).
12 M. Brion, G. Berthon and J. B. Fourtillan, *Inorg. Chim. Acta, 55,* 47 (1981).
13 G. Berthon, M. Brion and L. Lambs, *J. Inorg. Biochem., 19,* 1 (1983).
14 L. Lambs, M. Brion and G. Berthon, *Agents Actions, 14,* 743 (1984).
15 M. Schach von Wittenau, *Chemotherapy, 13,* 41 (1968).
16 J. L. Colaizzi and P. R. Klink, *J. Pharm. Sci., 58,* 1184 (1969).
17 S. T. Day, W. G. Crouthamel, L. C. Martinelli and J. K. H. Ma, *J. Pharm. Sci., 67,* 1518 (1978).
18 S. R. Martin, *Biophys. Chem., 10,* 319 (1979).
19 M. E. Dockter and J. A. Magnuson, *J. Supramol. Struct., 2,* 32 (1974).
20 F. J. C. Rossotti and H. Rossotti, *J. Chem. Educ., 42,* 375 (1965).
21 A. Sabatini, A. Vacca and P. Gans, *Talanta, 21,* 53 (1974).
22 A. M. Corrie, G. K. R. Makar, M. L. D. Touche and D. R. Williams, *J. Chem. Soc., Dalton Trans., 105* (1975).
23 A. Kayali and G. Berthon, *Polyhedron, 1,* 371 (1982).
24 M. J. Blais and G. Berthon, *J. Chem. Soc., Dalton Trans.,* 1803 (1982).
25 L. Lambs, *Thèse de 3ème Cycle, No. 950,* Poitiers, 1983.
26 L. J. Leeson, J. E. Krueger and R. A. Nash, *Tetrahedron Lett., 18,* 1155 (1963).
27 C. R. Stephens, K. Murai, K. J. Brunings and R. B. Woodward, *J. Am. Chem. Soc., 78,* 4155 (1956).
28 J. T. Doluisio and A. N. Martin, *J. Med. Chem., 6,* 16 (1963).
29 P. M. May, P. W. Linder and D. R. Williams, *J. Chem. Soc., Dalton Trans.,* 588 (1977).
30 G. Berthon, P. M. May and D. R. Williams, *J. Chem. Soc., Dalton Trans.,* 1433 (1978).
31 G. Berthon, C. Matuchansky and P. M. May, *J. Inorg. Biochem., 13,* 63 (1980).
32 G. Berthon, M. J. Blais, M. Piktas and K. Houngbossa, *J. Inorg. Biochem., 20,* 113 (1984).