Metal Ion–Tetracycline Interactions in Biological Fluids. Part 7. Quantitative Investigation of Methacycline Complexes with Ca(II), Mg(II), Cu(II) and Zn(II) Ions and Assessment of their Biological Significance

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Abstract

Previous studies based on computer-simulated distributions of several tetracyclines in blood plasma during treatment have revealed that the fraction of drug not bound to proteins almost exclusively occurs in the form of calcium and magnesium complexes. In contrast to former thoughts, it thus appeared that the bioavailability of tetracyclines should primarily depend on the physicochemical properties of the most predominant of these species rather than on those of the free parent molecules. In particular, the possible formation of electrically charged homo- or heterobinuclear complexes with the above two metals at the expense of their neutral diffusible mononuclear homologues should notably reduce the bioavailability of the drug.

The substitution of electron-attracting groups at positions 5–7, which tends to weaken the electron density of the phenolic diketone moiety, should help to prevent the formation of such binuclear complexes. This hypothesis recently proved valid for chlortetracycline but not for demethylchlortetracycline. Before future developments involving other possible structural changes can be envisaged, the present paper reports an investigation of the coordination of methacycline with Ca(II), Mg(II), Zn(II) and Cu(II) ions, the influence of the methylene group at C6 being expected to resemble that of the chlorine at position 7.

Corresponding results show that the methylene substituent does effectively prevent methacycline from generating binuclear complexes with calcium and magnesium, and its effect on copper and zinc coordination is similar. Potential biological implications are discussed on the basis of pertinent computer simulations.

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Introduction

The affinity of tetracyclines for metal ions was discovered many years ago [1, 2] and was expected to entail significant effects *in vivo* [3]. It was then largely confirmed that metal ions do interfere with many biological processes in which these antibiotics are involved. For example, diverse stages of the antibacterial activity of tetracyclines are thought to be linked to metal ion dependent mechanisms [3–7]. The deleterious impact of these drugs on osteogenesis [8], which may imply both collagen synthesis inhibition [9] and impairment of the mineralizing process itself [10], has been shown to result from their interactions with Fe(II) and Ca(II), respectively. The affinity of tetracyclines for calcium is also involved in their staining of teeth [11]. Gastro-intestinal absorption is a determining factor of the overall bioavailability of a drug, and that of tetracyclines is greatly influenced, usually negatively, by the simultaneous occurrence of metal ions in the gastrointestinal tract [12–16]. Reciprocally, the absorption of essential metal ions may be impaired in patients undergoing tetracycline therapy [17, 18].

In spite of several attempts to rationalize these clinical observations [6, 19–21], it was not until the advent of high speed computer programmes, allowing sophisticated analyses of coordination equilibria as well as simulations of large equilibrium systems, that the stoichiometries and concentrations of the different metal ion complexes formed by tetracyclines *in vivo* could be assessed on a quantitative basis. Thus, it was established in the first two parts of this series [22, 23] that, contrary to a still widely accepted principle [24], the so-called 'free' fraction of tetracyclines [25] not bound to proteins in the plasma of patients under treatment was actually present in the form of calcium [22] and, to a lesser degree, magnesium [23] complexes.

Accordingly, it was inferred from the above studies that the capacity of a tetracycline derivative to diffuse through tissue membranes towards its

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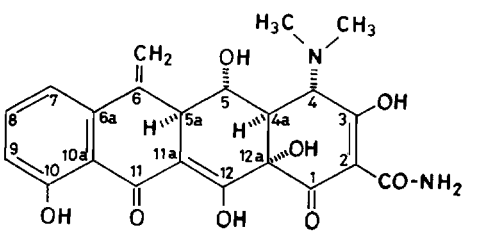
therapeutic target should no longer be appraised on the sole basis of the lipophilic properties of its free molecule [25–27], but should first be discussed in terms of the nature and electrical charge of its predominant complexes. More precisely, the fact that some tetracycline derivatives allow the formation of homo- or heterobinuclear complexes with calcium and magnesium whereas others do not should be considered, at least in a first approach, as an argument in favour of better bioavailability for the latter [28]. Clearly, given the metal-to-ligand ratios pertaining to blood plasma, possible electrically charged binuclear complexes tend to take up a predominant position in the distribution of the drug at the expense of neutral diffusible mononuclear species [22, 23, 28].

At first sight, to impose structural variations on the parent tetracycline molecule would appear a logical way of inducing the preferential formation of either type of complexes at will. Unfortunately, given that the minimal structural equipment represented by the 6-demethyl-6-deoxytetracycline is necessary to the antibacterial activity of tetracyclines *in vivo* [29], the possibilities for such variations are actually confined to the C5–C9 positions. Nevertheless, due to the complexity and to the conformational flexibility of the parent molecule [30], apparently minor substituent variations in these positions result in spectacular effects on the physicochemical, antibacterial and coordinative properties as well [29]. For example, the substitution of an electron-attracting group at C7 is likely to weaken the electron density of the diketophenolic moiety. As this site is thought to be involved in the coordination of calcium [7, 19, 20, 30–32] as well as magnesium [7, 30, 31] in aqueous solution, partially blocking its donor potentialities should lessen the capacity of the resulting derivative to generate binuclear complexes. This possibility was tested for the chloro substituent in the coordination of chlortetracycline (CTC) and demethylchlortetracycline (DMC) with calcium and magnesium [33]. The expected effect was found to be determining for CTC, but not significant for DMC [33], which illustrates well the above-mentioned complexity of this type of molecule.

In methacycline (MTC: see I), the methylene group also acts as an attractor for the electrons of the C10–C12 system [29], whose affinity for metal ions



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should logically be reduced. As a test of this hypothesis, and before other possible structural changes can be envisaged, the present paper reports an investigation of the coordination of MTC with Ca(II), Mg(II), Cu(II) and Zn(II) ions. The biological significance of the corresponding complexes has then been assessed and discussed using relevant computer simulations.

Experimental

Formation Constant Determinations

Reagents

The sample of methacycline hydrochloride was kindly supplied by Pfizer Laboratories. It was analysed for its antibiotic and hydrochloric acid contents through the appropriate Gran titrations and proved to be sufficiently reliable to be used without further purification. On account of the well documented instability of tetracyclines in aqueous media [30, 34], fresh solutions were frequently prepared and systematically assayed before use. Both original product and solutions were constantly kept in the dark under an atmosphere of purified nitrogen.

Stock solutions of calcium, magnesium, copper and zinc chlorides were prepared and standardized as described previously [22, 23, 35, 36]. Carbonate-free sodium hydroxide solutions were prepared by diluting BDH standard volumetric concentrates in doubly deionised freshly boiled water. Alkali titre and absence of carbonate were periodically checked by means of appropriate Gran plots [37] using Merck *pro analysi* potassium hydrogenphthalate.

Technique and experimental conditions

Potentiometric titrations were carried out using electrochemical cells of the following type

Glass electrode|methacycline, (M^{2+}), NaCl 0.15 mol dm^{-3}|NaCl sat.|Hg_2Cl_2–Hg

The technical equipment based on a Beckman Model 4500 mV meter was similar to that used in our previous studies in this series [35, 36]. Beckman glass and Calomel electrodes were fitted to an Ingold reaction cell thermostatted at 37 ± 0.02 °C, in which a nitrogen atmosphere was maintained by bubbling the gas into the solution. The electrode system was calibrated in the concentration scale, which implies that the pH notation will stand for –log [H] throughout.

Titrated solutions containing metal and methacycline were initially made sufficiently acidic to ensure that all donor groups of the latter were significantly protonated at the outset of each experiment. Within the limits imposed by the poor solubility of the antibiotic, metal-to-ligand ratios were varied to

TABLE I. Summary of the Titration Data Used for Calculating Formation Constants[a]

System	C_L	C_M	C_H	C_{OH}	pH range	N
Proton–methacycline	1.000		1.760	99.9	2.88–10.16	33
	1.942		3.561	99.9	2.63–10.25	55
	4.890		9.000	99.9	2.30–10.31	62
Calcium–methacycline	0.991	0.100	2.000	9.96	2.85–7.48	38
	0.991	0.150	2.001	9.96	2.53–6.50	32
	0.991	0.150	2.001	9.96	2.54–7.46	48
	0.991	0.251	2.016	9.96	2.83–7.29	53
	0.991	0.501	2.043	9.96	2.82–6.34	37
	0.991	1.002	2.097	9.96	2.80–5.72	29
	0.991	2.004	2.206	9.96	2.78–5.45	40
	0.495	1.002	1.103	9.96	3.05–5.99	37
Magnesium–methacycline	0.996	0.100	1.987	10.20	2.83–7.26	45
	0.996	0.150	1.993	10.20	2.83–7.19	45
	0.996	0.251	2.005	10.20	2.83–6.92	47
	0.996	0.501	2.035	10.20	2.82–6.88	68
	0.996	1.002	2.094	10.20	2.81–6.88	80
	0.996	2.004	2.214	10.20	2.77–9.60	102
	0.498	1.002	1.107	10.20	3.05–8.48	79
Copper–methacycline	1.000	0.101	2.011	10.03	2.81–8.20	51
	1.000	0.151	2.016	10.03	2.79–7.59	46
	1.000	0.252	2.027	10.03	2.78–6.57	47
	1.000	0.503	2.065	10.03	2.71–4.85	34
	1.000	1.006	2.109	10.03	2.66–4.44	31
	1.000	2.012	2.219	10.03	2.61–4.73	33
Zinc–methacycline	0.995	0.101	1.973	10.03	2.50–8.68	40
	0.995	0.152	1.978	10.03	2.83–9.12	39
	0.995	0.254	1.989	10.03	2.82–8.69	37
	0.995	0.508	2.016	10.03	2.82–8.34	43
	0.995	1.015	2.071	10.03	2.80–7.30	47
	0.995	2.030	2.179	10.03	2.76–6.89	45

[a]The initial total concentrations of methacycline (C_L), metal ion (C_M) and mineral acid (C_H) in the titrate as well as the sodium hydroxide concentrations (C_{OH}) in the titrant are expressed in mmol dm^{-3}; the pH notation stands for $-\log [H]$ (see text) and N represents the number of experimental observations in each titration.

the greatest extent possible over the set of experiments performed for each system (see Table I). As is commonly the case for tetracyclines, a slight opacity as well as foaming progressively occurred as the pH was raised, especially with calcium. Nevertheless, titrations were pursued until a steady drift was noted in the potential readings, this being considered as indicative of a precipitation process.

Calculation procedures

Following our usual approach [38], stoichiometries and stability constant estimates relative to the species possibly present in a given system were deduced from the shapes of pertinent protonation and complex formation curves. The MINIQUAD program [39] was used to refine these constants, and the final discrimination of the 'best' set was based on graphical comparisons using PSEUDOPLOT [40] and ESTA [41] simulations.

As outlined in the introduction, calcium and magnesium are present in far higher concentrations than tetracyclines in blood plasma, and so they tend to form predominant binuclear complexes whenever the ligand structure is favourable. In view of this, such species were specifically sought by investigating experiments that incorporated metal-to-ligand ratios equal to 2 separately [22, 23].

Results and Discussion

The protonation constants for methacycline and the formation constants for its interactions with calcium, magnesium, copper and zinc are shown in Table II. Figures 1 and 2 are given as typical examples of the above-mentioned approach.

Concerning the effect expected from the methylene group on the coordinative capacities of the

TABLE II. Formation Constants for the Complexes of Calcium(II), Magnesium(II), Copper(II) and Zinc(II) Ions with Methacycline at 37 °C in Aqueous Medium NaCl 0.15 mol dm^{-3} [a]

System	*p*	*q*	*r*	log β	±	*S*	*R*	*n*
Proton–methacycline	1	0	1	8.731	0.004	1.03E–7	0.00364	150
	1	0	2	15.810	0.006			
	1	0	3	18.783	0.009			
Calcium–methacycline	2	1	2	24.860	0.026	8.03E–9	0.00427	163
	1	1	0	5.306	0.037			
Magnesium–methacycline	2	1	2	25.196	0.014	1.52E–8	0.00398	357
	2	1	1	17.233	0.072			
	1	1	1	12.373	0.015			
	1	1	0	5.142	0.006			
Copper–methacycline	2	1	4	39.133	0.125	9.29E–9	0.00251	242
	2	1	3	35.891	0.069			
	1	1	2	20.282	0.035			
	2	1	2	31.094	0.052			
	2	1	1	23.751	0.065			
	1	1	1	17.268	0.027			
Zinc–methacycline	2	1	2	26.914	0.026	2.43E–8	0.00612	215
	2	1	1	19.517	0.048			
	1	1	1	13.425	0.022			
	1	1	0	6.660	0.013			

[a]The general formula of a complex is $M_qL_pH_r$; *S* = sum of squared residuals; *R* = *R* factor as defined in ref. 39; *n* = number of experimental observations (this number may be lower than the sum of observations corresponding to each system as indicated in Table I; this stems from the fact that experimental points obtained in the acidic pH range where metal complex formation is insignificant have eventually been excluded from MINIQUAD calculations).

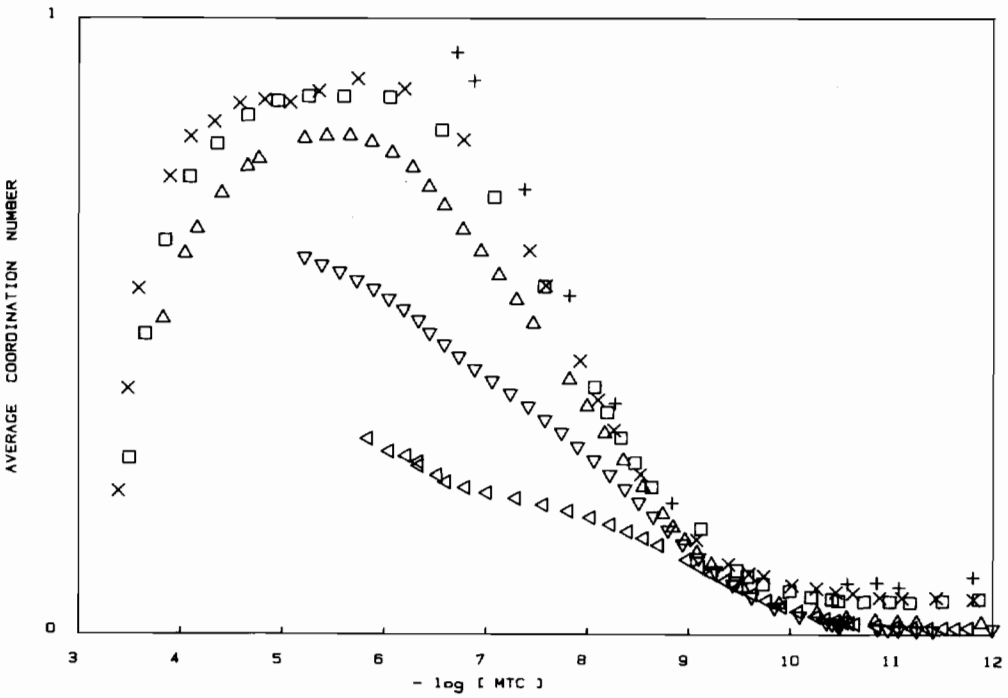

Fig. 1. Experimental formation curve for the zinc–methacycline system. The following symbols are in the respective order of the experiments as given in Table I: +, ×, □, △, ▽, ◁.

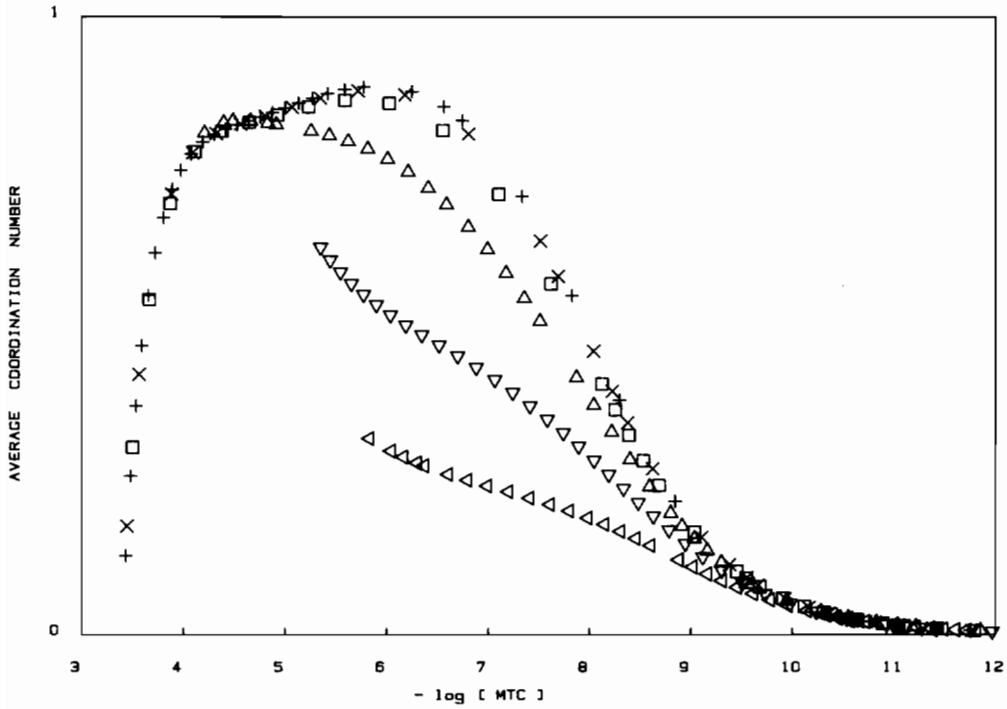

Fig. 2. Simulated formation curve for the zinc–methacycline system, as based on the results shown in Table II. Key to symbols as in Fig. 1.

diketophenolic system of methacycline, protonation constants may offer interesting grounds for preliminary discussion. According to the reference work by Leeson *et al.* [42] on the assignment of the acidity constants of tetracyclines, the most basic protonation step refers to the C4 dimethylamino group, the intermediate is associated with the C10–C12 phenolic diketone system, and the most acidic is related to the C1–C3 tricarbonylmethane moiety. Among the various tetracyclines investigated in the previous parts of this series [22, 33], doxycycline (DOXY) may be considered as the most appropriate substance to which MTC may be compared since the only difference between these two molecules consists in the nature of their substituent in C6, *i.e.* methyl and methylene respectively. If we consider the step-stability constants relative to the three sites above, it is remarkable that the protonation of the C4 dimethylamino group is not affected at all by the nature of the substituent at C6 since the values relative to MTC (8.73) and DOXY (8.68) are very similar. In contrast, the constant for the second protonation step of MTC (7.08) is significantly lower than that of DOXY (7.41). Finally, the constants relative to the protonation of the tricarbonyl methane system are again of the same order of magnitude (2.97 and 3.10 respectively). It may be inferred from the above that the transmission of the electron-attracting effect of the methylene group from C6 to C11 through the conjugative resonance of the C ring effectively lowers the electron density of the C10–C12 system.

Moreover, if we comparatively examine (i) the above effect of the methylene group at C6 of MTC with respect to DOXY, and (ii) the previously investigated [33] effect of the chlorine atom at C7 of CTC with respect to tetracycline (TC), it seems that these two substituents in their different positions induce distinct types of interactions for the protonations of the related tetracyclines. As was pointed out above, only the diketophenolic system is affected in the protonation of MTC, whereas both the C4 dimethylamino group and the C10–C12 system undergo significant changes in the protonation of CTC. This suggests that the effect of the methylene group mainly reflects its electron-attracting influence, while the chloro substituent is also involved in the interactions of CTC with water near the C4–C7 positions. Incidentally, the lower protonation constant of the C10–C12 system of DMC with respect to that of CTC [33] may now be interpreted as being due to the absence of the methyl group in C6 (+*I* inductive effect) with respect to both TC and CTC.

As was the case for protonation equilibria, the formation of calcium and magnesium complexes with MTC may first be analysed in terms of a comparison with the results obtained on DOXY [22, 23]. Concerning this, a marked similarity can be observed between complex stoichiometries in homologous

systems, apart from the fact that, as originally expected, no binuclear complex is formed by MTC. Indeed, calcium only forms ML and ML_2H_2 with MTC, whereas it also gives rise to M_2L (and MLH) with DOXY [22]. Similarly, magnesium complex stoichiometries with MTC and DOXY are exactly the same except that the M_2L DOXY species is replaced by ML with MTC. It is also of interest to note that complexes of identical stoichiometries are systematically less stable with MTC than with DOXY. If, on the basis of the observations made on protonation equilibria, we postulate that the diketophenolic system is the only coordinative group to be affected by the structural differences between MTC and DOXY, the above decrease of stability seems to imply that this site is involved in all corresponding complexes. This is in line with previous authors' conclusions according to which the first calcium ion [7, 19, 20, 30–32] as well as the first magnesium ion [7, 30, 31] to bind a tetracycline molecule when the pH is raised would be coordinated through the C10–C12 system. However, this hypothesis appears to be to some extent contradictory with the expectation that the absence of binuclear complexes of calcium and magnesium with MTC would result from the limitation of the coordinative capacities of the diketophenolic moiety. Moreover, considering the increments of stability due to the fixation of successive protons within the magnesium complex series also leads to question the above-mentioned conclusions: (i) the increase in stability observed from ML to MLH (7.23) closely corresponds to the protonation of the diketophenolic system (see above), which seems to imply that this system may not be involved in the formation of MLH; (ii) in the same manner, the fact that the increment in stability between ML_2H and ML_2H_2 (7.96) represents half the step between the two micro-protonation constants referring to the C4 dimethylamino group and the C10–C12 moiety suggests that half the fraction of LH involved in metal coordination would not bind magnesium through its diketophenolic group. These structural problems will be studied in more detail in the next parts of this series.

Returning to the comparison between the effects of the chlorine at C7 and the methylene group at C6, it is worth reasserting that the influence of the methylene substituent seems to be essentially due to its electron-attracting capacity. As will be seen in a next part of this series [43], the prime effect of the chlorine at C7 on CTC metal coordination stems from the distortion that its antagonism with the methyl substituent induces between positions 6 and 7, which makes the C–O bonds of the C10–C12 moiety point in opposite directions, thereby preventing them from binding calcium or magnesium. This will explain why DMC, which has no methyl group at C6, can still form M_2L complexes with these two metals, whereas CTC cannot. In the present case, no such conformational effect is possible, and it can thus be concluded that the absence of M_2L is due to the electronic influence of the methylene group.

For copper complex equilibria, the shift of the protonation curves over $\bar{r} = 2$ towards more acidic pH values in the presence of metal is characteristic of the formation of complexes with the diprotonated form of MTC. This implies that 1:2 metal-to-ligand ratio complexes may involve MTC in any of its three protonation states. Accordingly, various sets of constants combining MLH_2, MLH, ML, but also ML_2H_4, ML_2H_2, ML_2, ML_2H_3 and ML_2H were refined in a first approach. Then, M_2L and M_2LH were introduced in the calculations to test the possible presence of binuclear species. The choice of a 'best' set of constants proved to be particularly difficult since different combinations produced almost equivalent numerical *and* graphical fits.

As protonation curves in the presence of copper tend to meet the reference curve without metal near $\bar{r} = 1$ (not shown here), advantage was taken of this specific feature to help in understanding the formation of acidic complexes. An additional graphical approach was thus used, in which the number of dissociable protons (NDP) of MTC was arbitrarily taken as equal to 1. In such a case [44], the first protonation constant is neglected and LH is considered as L^*. Accordingly, parts of formation curves interpretable in terms of L^* provide information on LH complex equilibria which cannot be disclosed on normal formation curves drawn as a function of L. For example, two points were confirmed in the present case: (i) copper complexes of the diprotonated form of MTC do exist, and (ii) the maximum coordination number of copper with LH is 2. The deprotonation curves defined in the QBAR task of the ESTA library [41] and already described by other authors [45] were also used. Finally, the set of constants shown in Table II were considered as the most realistic on both numerical and graphical grounds. Concerning this, it should be noted that although it was not rejected during MINIQUAD refinements, the ML species was eventually discarded since (i) its percentage never reached more than 2% throughout all experiments in Table I, and (ii) the standard deviation affecting its constant was usually greater than this constant itself (found to be equal to 10.40).

Among all the tetracyclines investigated so far [36], methacycline appears to give rise to the most numerous copper complexes, all of them being protonated. If we examine the increments in stability relative to the different protonation steps involved in these complexes, it is interesting to note that the increase between MLH and MLH_2 (3.01) corresponds to the protonation of the tricarbonylmethane moiety (2.97). This suggests that the copper(II) ion present

in MLH is bound either to the C10–C12 system or to the C4 dimethylamino group, which is in agreement with previous conclusions [5]. Nevertheless, to interpret the successive increments in the series ML_2H–ML_2H_4 is less straightforward: (i) the fact that the transition from ML_2H to ML_2H_2 (7.34) roughly corresponds to the protonation of the diketophenolic system (7.08) implies that the L form of MTC coordinates copper through its C4 dimethylamino group; (ii) as was the case for the protonation step from MLH to MLH_2, the transition from ML_2H_3 to ML_2H_4 (3.24) corresponds to the addition of a proton onto the C1–C3 moiety (2.97), which again suggests that the LH form of MTC may bind copper through either of its other two donor groups; and (iii) the increase in stability between ML_2H_2 and ML_2H_3 (4.80), which represents about half the step between the individual protonation constants relative to the tricarbonylmethane moiety and the diketophenolic system, is less easy to understand: the only hypothesis that can be inferred from this observation is that one of the MTC units in the ML_2H_2 species is probably bound to copper through its C10–C12 system.

Concerning zinc–MTC equilibria, the main point of interest is that no complex is formed with the diprotonated form of the ligand, which differs from the results previously obtained on DOXY [35]. This result is difficult to explain on the basis of only formation constants, more especially since little information is available in the literature on zinc–tetracycline interactions [1, 2, 5]. It may nevertheless be of interest to point out that the same observation was also made for CTC and DMC [35].

As far as possible coordination sites are concerned, the fact that the increments in stability due to the protonation of ML (6.76) as well as ML_2H (7.40) – into respectively MLH and ML_2H_2 – are observed to be near that of the diketophenolic system suggests that the C4 dimethylamino group is involved in the binding of zinc to the L form of MTC.

Computer Simulation Studies

After testing the expected incapacity of MTC to form binuclear complexes with calcium and magnesium, the main objective of the present paper was to assess the consequences of the non-existence of such species on the distribution of MTC in blood plasma during treatment. By reference to our recent studies [35, 36], it was also of interest to investigate (i) the capacity of MTC to mobilise zinc and copper into their respective plasma low-molecular-weight (l.m.w.) fractions, and (ii) the extent to which each of the two above metals and MTC can affect the bioavailability of each other in the gastro-intestinal fluid under therapeutic conditions. Gastro-intestinal interactions of calcium and magnesium with MTC and other tetracyclines will be dealt with in a separate study.

Technical Considerations

Blood plasma simulations were performed with the help of the ECCLES program [46], using our current database [47] in which formation constants reported in Table II were incorporated beforehand. Mobilising effects of MTC with respect to zinc and copper were analysed by scanning the antibiotic concentration from 10^{-7} to 10^{-3} mol dm^{-3}: this largely encompasses the average therapeutic level of the drug that can be estimated near 10^{-5} mol dm^{-3} [29]. The distribution of MTC was monitored simultaneously.

Gastro-intestinal investigations were carried out using our NEUPLOT program [48], which enables one to analyse the percentage of the neutral fraction of a given reactant as a function of pH.

Blood Plasma Simulations

The most important result of these investigations concerns the extent to which calcium and magnesium ions condition the distribution of MTC at its average therapeutic concentration. As may be seen in Table III, electrically neutral complexes of MTC generated by these two metals represent about 80% of the fraction of drug not bound to plasma proteins (formerly considered to be free [25, 26]). This is by far the highest percentage of neutral drug ever found in plasma among all tetracyclines hitherto investigated [28, 33]. Indeed, the neutral metal-complexed fraction of CTC, second in decreasing order of importance, amounts to 40% only [33]. For the sake of comparison, it is also worth pointing out that the ML species with calcium represents more than 57% of MTC by itself.

In previous clinical studies carried out on man, it was shown by Kunin [50] that MTC is more slowly cleared by the kidneys than is the parent tetracycline (TC). This property, which was attributed to a more marked serum binding of MTC, would account for the higher and more sustained blood levels noted after oral as well as intravenous administration of this analogue [50]. Almost at the same time, other authors [25] confirmed the high serum binding of MTC with respect to TC in human subjects; MTC was also shown to exhibit a particularly strong interaction with dog serum proteins, but differences with TC proved less significant. In dogs, MTC gave considerably higher serum concentrations than the other tetracyclines, which, according to the above authors [25], 'cannot be explained by its strong interaction with serum proteins alone'. For example, 6-demethyl-6-desoxytetracycline (DSC), although as highly bound as MTC, gives much lower serum concentrations. These authors considered the 'free' drug concentration in serum (which actually represents the fraction of drug not bound to proteins) as about the same as the drug concentration in interstitial fluids. Then, taking into account (i) the high tissue concentration previously demonstrated for tetracyclines [51], and

TABLE III. Simulated Distribution of the Fraction of Methacyline (MTC) not Bound to Proteins in Blood Plasma During Treatment[a]

Species composition[b]	log β	Percentage[c]	Electrical charge
Ca–MTC	5.31	57.2	0
Mg–MTC	5.14	18.1	0
Mg–MTC–H	12.37	12.2	+1
MTC–H	8.73	5.4	−1
$MTC-(H)_2$	15.81	2.6	0
Ca–MTC–LTA–H	13.73[d]	1.1	0
Mg–MTC–LTA–H	13.80[d]	0.6	0
$Ca-MTC-PO_4-(H)_2$	25.38[d]	0.4	−1
$Mg-MTC-PO_4-(H)_2$	25.55[d]	0.2	−1
$Ca-(MTC)_2-(H)_2$	24.86	0.2	0
$Mg-(MTC)_2-(H)_2$	25.20	0.2	0
Ca–MTC–CTA–H	14.73[d]	0.2	−2

[a]The total concentration of antibiotic is taken as 1.0×10^{-5} mol dm^{-3} [29]. Free metal concentrations are the same as in ref. 55. Total concentrations of naturally occurring l.m.w. ligands are taken from ref. 46. pH is fixed at 7.40. [b]LTA = lactate, PO_4 = phosphate, CTA = citrate. [c]Percentages lower than 0.2 are not reported. [d]Constants evaluated from statistical considerations.

TABLE IV. Capacity of Methacycline (MTC) to Mobilise Copper(II) and Zinc(II) Ions into their l.m.w. Fractions in Blood Plasma, as Assessed by Computer Simulations[a]

Metal ion	Plasma concentration[b] of MTC (mol dm^{-3})	Percentage of the l.m.w. metal mobilised by MTC	log P.M.I.
Copper(II)	1.00×10^{-5}	0.1	0.00
	3.16×10^{-5}	0.3	0.00
	1.00×10^{-4}	1.0	0.00
	3.16×10^{-4}	3.2	0.01
	1.00×10^{-3}	9.0	0.04
Zinc(II)	3.16×10^{-6}	0.1	0.00
	1.00×10^{-5}	0.3	0.00
	3.16×10^{-5}	1.1	0.00
	1.00×10^{-4}	4.3	0.02
	3.16×10^{-4}	12.7	0.06
	1.00×10^{-3}	30.4	0.16

[a]The plasma mobilising index (P.M.I.) is defined in the text. [b]MTC scanned concentrations lower than 1.00×10^{-5} mol dm^{-3} are not mentioned when the corresponding metal mobilised fractions are inferior to 0.1%.

(ii) the low free drug concentrations found in their own study, they proposed that: 'most of an intravenously administered tetracycline is reversibly bound in the tissue depots and smaller quantities are in the aqueous phase such as interstitial fluid'; that 'serum proteins are a depot of rather low capacity'; and that 'the distribution of a tetracycline is therefore primarily influenced by its affinity for body tissues' [25]. Such a rationale would account for the highest so-called 'free' serum concentration of OTC, the least serum protein-bound as well as the least lipoid-soluble tetracycline. Similarly, with respect to its high total serum concentration, the comparatively low extent of the fraction of MTC not bound to proteins would thus derive from both protein binding *and* tissue affinity. In the above studies [25], the half-life of MTC was also found to be the highest of all the tetracyclines examined. Our present results correlate well with these clinical observations: the very high neutral fraction of MTC expected to occur in plasma (Table III) should logically favour the tissue diffusion and lower the renal clearance of the drug.

As far as MTC interactions with zinc and copper are concerned, Table IV reports the variations of the l.m.w. fraction of each of these metals within the limits of drug concentrations being scanned. The P.M.I. index, which expresses the ratio by which the l.m.w. fraction of a metal is changed in the presence of a drug with respect to the normal state, was also calculated. Clearly, it is unlikely that MTC can

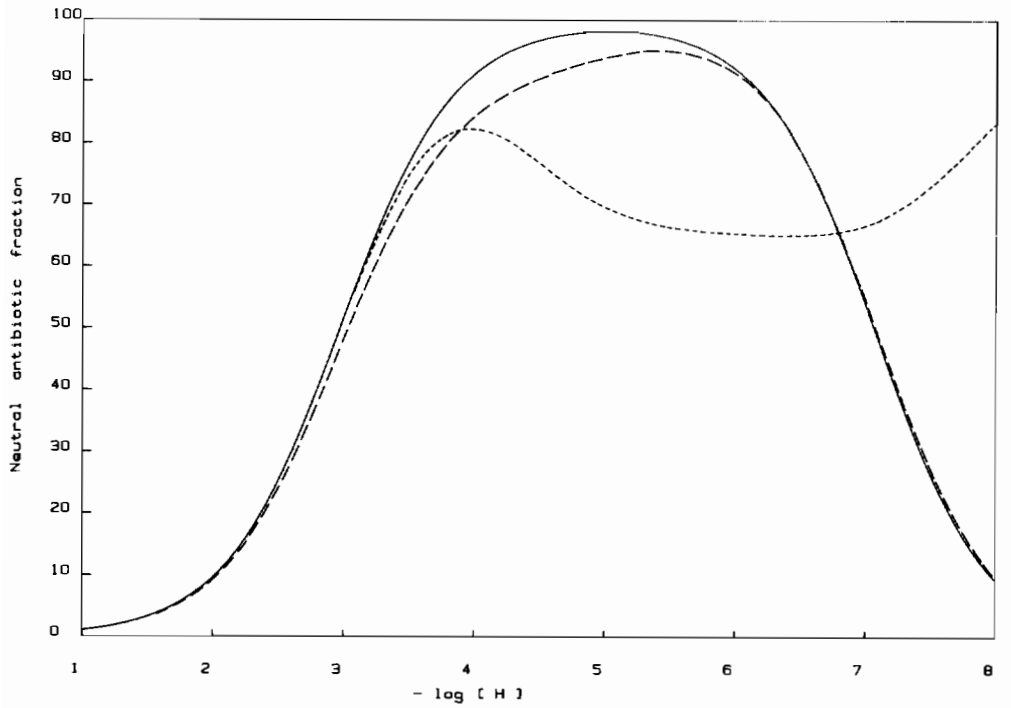

Fig. 3. Variations of the electrically neutral fraction of methacycline alone (——) and in the presence of copper (— — —) and zinc (- - - -) as a function of pH under gastro-intestinal conditions.

quantitatively interfere with the distribution of zinc and copper under therapeutic conditions. Nevertheless, it should be borne in mind that, depending on their lipophilic properties, neutral complexes can be biologically active even at seemingly negligible concentrations [49]. With respect to this, further studies would be necessary to assess to what extent protonated ternary MTC complexes of copper as well as zinc with histidine and other monoanionic amino acids could favour the tissue diffusion of these two metals.

It should finally be mentioned that, given their very low free concentrations with regard to those of calcium and magnesium, copper and zinc cannot exert any significant influence on the distribution of the antibiotic in blood plasma.

Gastro-intestinal Interactions

As outlined in the introduction, the overall bioavailability of tetracyclines is frequently impaired by the simultaneous presence of metal ions in the gastro-intestinal tract. First of all, the pH rise due to the administration of antacids may result in reduced solubility, hence a poorer absorption of these antibiotics [14, 52]. Nevertheless, since the latter process is a passive diffusion phenomenon [53], tetracycline bioavailabilities are mainly influenced by the physicochemical properties of the metal complexes that prevail in the gastro-intestinal fluid [12, 16, 17, 54]. This has been confirmed by the fact that: (i) the effects observed depend on the nature of the metal salt [13–15]; (ii) the absorption of the metal ion is reciprocally impaired [15, 17, 18]; and (iii) clinical observations were recently substantiated by simulations based on corresponding equilibrium data [35]. Among these properties, electrical charges are the most important factor to be taken into account since neutral species alone are likely to dissolve into phospholipid cell membranes.

For reasons developed above, it is generally advised to administer tetracyclines separately from meals. This is also the case for trace metals such as copper [56] and zinc [57], whose bioavailability is reduced in the presence of food. It was thus of interest to quantitate the possible interactions between MTC and each of these metals in an empty stomach. Concentrations of copper and zinc were taken as corresponding to respective doses of 2 mg [56] and 45 mg [57] of each element administered in 200 cm^3 of water. The concentration of MTC was considered as 3.13×10^{-3} mol dm^{-3}, corresponding to the administration of the usual dose of 300 mg of MTC hydrochloride in the same volume of water.

Figure 3 shows the influence of copper and zinc on the distribution of MTC in the gastro-intestinal fluid under the above conditions. Given the low concentration of copper with respect to that of the antibiotic, no significant effect is to be expected from this metal on the bioavailability of the drug. This is no longer the case for zinc, which lowers the fraction

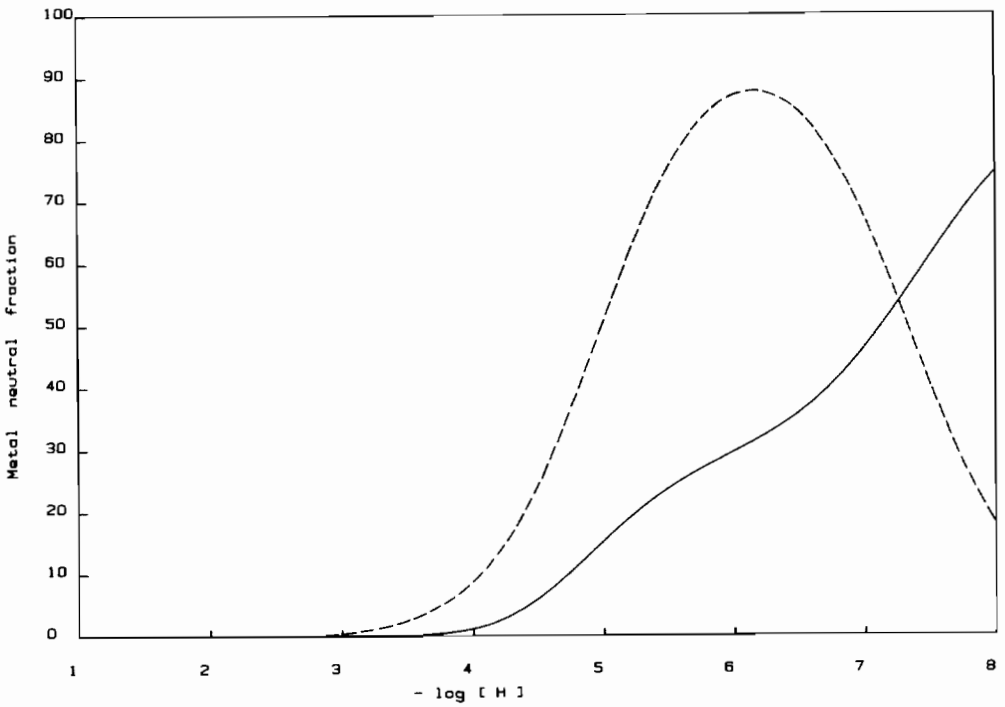

Fig. 4. Influence of methacycline on the bioavailability of copper (– – –) and zinc (——) in the gastro-intestinal fluid.

of neutral drug between pH 5 and 6 but tends to maintain it at approximately the same level up to pH 8 and above. One can expect from this observation that zinc may slightly reduce the bioavailability of MTC in the proximal part of the small intestine but rather enhance it in its distal fraction.

Due to its lower concentration and to the higher stability of its complexes, copper is more affected than zinc by the presence of MTC in the gastro-intestinal fluid. In particular, the remaining free fraction of copper is logically less than that of zinc. Its neutral fraction reaches more than 80% near pH 6.5 (Fig. 4), which coincides with duodenal conditions, and is still of the order of 20% at pH 8. MTC should thus favour copper bioavailability throughout the whole small intestine, its effect being maximum in the duodenum. Although less important, its influence on zinc is also quite significant since more than 30% of zinc is present as neutral species near pH 6.5. In contrast to copper, the mobilisation of zinc by MTC increases with pH, and its bioavailability should thus be more favoured in the distal part of the small intestine. Concerning these interactions, it is worth recalling that the more lipophilic a neutral species is, the more likely it is also to precipitate in aqueous media. It is thus advisable to put the above considerations in perspective with the experimental conditions reported in Table I.

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