

almost totally compensated for by a correlative decrease in the concentration of the cysteinate ternary complex above [15].

Our latest investigation on the subject deals with the formation of ternary complexes of zinc and histamine with a series of dicarboxylic acids. Indeed, these substances are likely to meet the following requirements: (i) to have a low affinity for zinc on their own, (ii) to induce the formation of stable ternary complexes of this metal with histamine. In this respect, ligands with O donors are well known to form particularly stable mixed-ligand complexes of 3d metal ions with aromatic amines [16], especially those including an imidazole moiety [17].

Among oxalate (studied as a reference), fumarate, succinate and malate, only the latter can be expected to promote a significant increase of the neutral metal-complexed fraction of histamine in blood plasma.

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PS6.12 — TU

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DO TETRACYCLINES HAVE ANY INFLUENCE ON ZINC AND COPPER BIOAVAILABILITIES AT BLOOD THERAPEUTIC LEVELS?

Growing attention has recently been paid to the possible interference of organic pharmaceuticals with essential trace metal bioavailabilities [1-3]. Indeed, a vast majority of these substances contain donor groups likely to bind metal ions to such an extent that the normal distribution of the latter may be significantly upset. This may either lead to the expected activity of the drug or result in the occurrence of undesirable side effects [1-5]. As far as tetracyclines are concerned, their interactions with metal ions have been reported to play a critical part in important biological processes such as their deleterious impact on mineralizing tissues [6], their antibacterial activity [7] and their gastrointestinal absorption [8].

Analysing these interactions on a quantitative basis is not straightforward since, (i) account being taken of both antibiotic therapeutic doses and trace metal levels occurring *in vivo*, the concentrations of the complexes formed by tetracycline derivatives are very low, (ii) attempts at concentrating these species would upset their labile equilibria, hence their particular distribution in the biological fluid. In such cases, the only available investigation technique consists of the use of computer models which permit to simulate the distribution of all the coexisting complexes. Such models [9-11] are built up from (i) the analytically measurable overall concentrations of the reac-

tants, (ii) the parameters which relate these overall concentrations to the individual concentrations of all the species present in the medium, *i.e.* the corresponding stability constants determined beforehand under suitable experimental conditions.

This kind of technique has already been used for analysing the influence of the interactions between calcium, magnesium and various tetracyclines on the bioavailability of these antibiotics in blood plasma [12-15].

Zinc and copper are essential trace metals whose properties may be related to bacterial infection and its consequences. Zinc is the necessary cofactor of many enzymes involved in the biosynthesis of proteins and nucleic acids [16], whereas copper is known to exert antiinflammatory effects [17]. Besides, these two metals have been reported to be directly involved in tetracycline activity. The antagonistic role played by zinc ions against the gastrointestinal absorption of tetracyclines is well documented [8,18]. Moreover, both zinc and copper would mediate the binding of tetracyclines to macromolecules, especially DNA [19,20].

The coordination of zinc(II) and copper(II) with tetracycline (TC) and five of its derivatives, namely oxytetracycline (OTC), doxycycline (DOXY), minocycline (MINO), chlorotetracycline (CTC) and demethylchlorotetracycline (DMC), was investigated by potentiometry under biological conditions of temperature and ionic strength (37°C, NaCl 0.15 M). Full details on these studies will be published elsewhere [21]. It is only worth mentioning that 26 and 24 complexes were characterized for zinc and copper, respectively.

The formation constants of the above-mentioned complexes were introduced into the blood plasma databank relative to the ECCLES simulation programme [9]. Then the plasma concentration of each antibiotic considered in succession was scanned between 1×10^{-7} M and 1×10^{-3} M, whereas the relative importance of each tetracycline-containing complex in the distribution of both zinc and copper was examined. These scanning limits were chosen so as to encompass the average therapeutic concentration of antibiotic in plasma, generally close to 1×10^{-5} M [12]. The PMI parameter expressing the evolution of the low-molecular-weight fraction of metal in the presence of drug with respect to normal plasma [22] was also monitored.

For the therapeutic concentration above, the percentages of the most predominant complexes of zinc or copper involving each tetracycline derivative in turn never reached 1%; they even revealed themselves quite negligible for DOXY, MINO and DMC. Accordingly, the corresponding log PMI values relative to zinc and copper were found to be nil for all tetracyclines. Moreover, the same parameter reached only 0.10 for zinc with DOXY, 0.09 for zinc with TC and CTC, and 0.14 for copper with OTC, at a concentration of 1×10^{-3} M of antibiotic.

No significant influence of any of the present tetracycline derivatives can thus be expected on zinc and copper bioavailabilities in blood plasma. Nevertheless, further work would be required to determine the stoichiometry of the complexes predominating in the intracellular fluid and to assess their significance.

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PS6.13 — TH

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LITHIUM TRANSPORT AND FACTORS AFFECTING THE MOVEMENT OF LITHIUM IN ISOLATED JEJUNAL MUCOSA OF GUINEA PIG

Lithium carbonate is widely used in the prophylactic treatment of manic-depressive psychoses [1] and is always administered orally. Lithium (Li^+) absorption from small intestine (Mucosal to Serosal) is passive both *in vitro* [2-4], and *in vivo* [5]. It is not affected by temperature, substrate depletion and metabolic inhibitors [6]. One criticism of these earlier studies is that a multicompartiment system with muscle and connective tissue is present and we now report studies in a three compartment system and also in isolated epithelial cells. Isolated jejunal mucosa of guinea pig was prepared and mounted to occlude a «porthole» separating two flux chambers [7]. Both sides were exposed to oxygenated Krebs-Tris buffer at 37°C.

Lithium replaced sodium, total $\text{Na}^+ + \text{Li}^+$ concentration being 106 mM.

Viability was tested by observation of active transport, potential difference, ^3H -PEG900 permeation and histological integrity. Bi-directional transfer of Li^+ across the epithelium was measured using stable isotopes ^6Li and ^7Li [8]. Lithium was determined using either an IL Video 22 or IL 357 atomic absorption spectrometer. Bi-directional fluxes of ^6Li and ^7Li showed no asymetry suggesting that there was no active component involved. Luminal and basolateral surfaces handled Li^+ isotopes similarly. Li^+ movement was independent of glucose transport and there appears to be no significant interaction between Li^+ and either Ca^{2+} or Mg^{2+} .

Acidification of the serosal side alone (pH 5.4) stimulated Li^+ absorption ($P < 0.01$) whereas mucosal acidification alone had no effect on transport. Neither treatment affected tissue uptake. Lithium, therefore, might be substituting for Na^+ in the Na^+/H^+ exchanger [9]. The pH gradient dependent increase in absorption was abolished by 1 mM Amiloride ($P < 0.0004$).

In further studies using ^3H -PEG900 as a measure of paracellular permeation, permeation of lithium correlated with that of PEG suggesting that movement of lithium in either direction occurred via the same PEG permeable, extracellular pathway. Confirmation for this route was obtained using solutions of high osmolarity, which collapsed the tight junctions [10]: lithium absorption was reduced ($P < 0.02$).

The transmucosal fluxes and tissue uptake of lithium in the absorptive (M to S) and secretory (S to M) directions were linearly related to the lithium concentration. Furthermore, the uni-directional fluxes both in the absorptive and secretory direction were similar. Total lithium transport after 45 minutes into serosal or mucosal compartments was 3-4 times greater than that found in the tissue. The plasma membrane of epithelial cells offers greater resistance to the movement of lithium than intact epithelium which suggests that the majority of ions pass via «pores» in the epithelium.