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Effect of taurine, L-glutamine and L-histidine addition in an amino acid glucose solution on the cellular bioavailability of zinc

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Radioactive zinc was used to study the effect of a binary parenteral nutrient solution, composed of amino acids and glucose, on zinc uptake by fibroblasts. The influence of addition of taurine, L-glutamine and of the increase in L-histidine content of the admixture was assessed. The pure mixture was highly toxic for cells and so it was diluted 1/5 in tyrode buffer with 2% albumin. As compared with cells incubated in the buffer containing albumin, zinc absorption was significantly higher ($P < 0.05$) in the presence of the amino acids of the mixture. Amino acids thus increased bioavailability by displacing zinc bound to albumin. When the histidine concentration in the nutrient medium (4.2 mM) was doubled, inhibition was noted after 30 min of incubation and zinc uptake thereafter remained comparable to that in histidine-free medium. The addition of glutamine (4.2 mM), usually not present in binary mixtures, resulted in significant differences as compared with glutamine-free control medium. Taurine (0.8 mM), led to a constant increase in zinc uptake by fibroblasts as compared with that obtained with taurine-free mixture. However, ultrafiltration showed that taurine was not able to displace zinc from albumin.

Keywords: taurine, L-glutamine, L-histidine, total parenteral nutrition, zinc, bioavailability, fibroblasts

Introduction

Considerable published work has shown that it is indispensible to supplement patients on parenteral nutrition with trace elements, since deficits can lead to serious long-term disorders. Patients on parenteral nutrition often present disorders resulting from zinc deficiency: primarily skin manifestations (dermatoses) (Arlette & Johnston 1981, Ortega *et al.* 1985) in which enteropathic acrodermatitis lesions are often observed (Weismann *et al.* 1983), accompanied by a number of biochemical disorders (Bates & McClain 1981). Even in the absence of clinical signs of deficiency, the trace element status of patients on total parenteral nutrition (TPN) is probably not optimal and undoubtedly affects their immune defenses (Ladefoged & Jarnum 1983), as well as inflammatory processes (Hull & Cassidy 1977). It is thus important to supply trace elements; however, relatively few data are available to enable a precise evaluation of the effects of other components in nutrient mixtures on the bioavailability of these trace elements. Clinical observations made during TPN in humans (Henkin 1977), as well as animal experimentation (Freeman & Taylor 1977), have shown the existence of trace element deficiencies and interactions between trace elements and amino acids. Actually, some amino acids may form chelates with zinc in blood (Kamoun *et al.* 1982). This complexation displaces zinc bound to albumin and provokes its elimination in urine. Subsequently, this complexation leads to zinc deficiency. However, while increasing urine excretion of zinc, certain amino acids may also facilitate cellular uptake of this element, increasing its bioavailability. Van Rij *et al.* (1975, 1979) reported these interactions between trace elements and amino acids *in vivo*. Cysteine (Cys) and L-histidine (His) apparently modify bioavailability during parenteral nutrition (Yunice *et al.* 1978).

The aim of this work was to determine the effect of a binary mixture, containing glucose and amino acids, on cellular bioavailability of zinc by measuring the amount of radioactive zinc incorporated by cultured human diploïd fibroblasts (MRC5). We then examined the influence of adding two amino acids, i.e. taurine (Tau) and L-glutamine (Gln), and of increasing the His level in the mixtures.

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Even though Tau and Gln are not usually included in TPN solutions, nutritionists have expressed the desire to add them to parenteral nutrition mixtures, since they play important biological role: Tau in the retina (Ament *et al.* 1986) and the brain (Thurston *et al.* 1980), and Gln in muscle tissue (Fürst *et al.* 1990). In addition, Tau apparently plays a detoxifying and antioxidant role in the organism (Gaull 1986). To our knowledge, no information is available concerning the interactions between Tau and trace elements such as zinc. The MRC5 cell line used has the advantage of being easily available and employed by many laboratories. Moreover, fibroblasts are ubiquitary cells found in the connective tissue of all organs of the body. Of course, further steps will be necessary to draw conclusions regarding applications to man.

Materials and methods

Nutrient mixture

The binary mixture (BM) was composed of a solution of amino acids (Azonutril® 25; Kabi Pharmacia, Paris, France) and of two glucose solutions: Glucose 50% (Aguettant, Lyon, France) and Glucose 30% (Delmas, Tours, France). The exact composition of the formula is listed in Table 1.

Cells

Experiments used MRC5 fibroblasts (ATCC CCL171, Flow Laboratories, Uxbridge, UK). Cells were grown in RPMI 1640 medium (Gibco, Grand Island, NY) containing 15% fetal calf serum (FCS) (Gibco), 2 mM L-glutamine (Gibco), 172 000 IU l^{-1} of penicillin G (Boehringer-Mannheim, Mannheim, Germany), 172 mg l^{-1} of streptomycin (Boehringer-Mannheim), 54 mg l^{-1} of kanamycin (Boehringer-Mannheim) and 0.5 mg l^{-1} of fungizone (Squibb, Princetown, NJ). Fibroblasts were incubated in culture bottles (Falcon®; Becton-Dickinson, Plymouth, UK) at 37 °C in sterile water-saturated atmosphere containing 6% CO_2 (incubator Forma Scientific, Marietta, OH). Tests were done between the 30th and 40th transfer.

Table 1. Composition of the basal nutrient admixture used

Ingredients	Volume (ml)	Concentration (mmol/l)
Azonutril 25	500	
L-Isoleucine		12.9
L-Leucine		35.3
L-Lysine acetate		31.7
L-Methionine		21.0
L-Phenylalanine		25.2
L-Threonine		13.9
L-Tryptophane		4.2
L-Valine		35.5
L-Alanine		35.5
L-Arginine		5.7
L-Glutamic acid		11.3
L-Histidine		10.7
L-Proline		23.1
L-Aspartic acid		10.0
L-Citrulline		5.7
L-Cysteine hydrochloride		2.8
Glycocolle		40.0
L-Ornithine acetate		5.9
L-Serine		4.1
L-Tyrosine		0.4
Neutral Na sulfite		0.19 g/l
Glucose 30%	750	1250
Glucose 50%	250	694

Choice of experimental conditions

BM was diluted 1/5 in tyrode buffer (Ty), pH 7.4, or in tyrode buffer 2% albumin (Ty-Alb), pH 7.4. These two dilution buffers had previously been used in the laboratory during the development of a technique for studying the short-term kinetics of zinc uptake by human skin fibroblasts (Guiraud *et al.* 1992). Solutions were filtered through 0.22 μm pore size membrane filters (Sartorius, Göttingen, Germany). pH and osmolarity were measured systematically. The zinc concentration was determined by atomic absorption spectrophotometry (model 560; Perkin Elmer, Norwalk, CT, equipped with an HGA 500 oven). After trypsinization, cells were dispensed in 35 mm diameter Petri dishes (Falcon®, Becton-Dickinson). Percent cell survival was determined at confluence by replacing the culture medium with BM/Ty and BM/Ty-Alb solutions, except in the case of control cells, which were maintained either in the buffers described above at 37 °C and 2.5% CO_2 or in the culture medium. After a 2 h incubation, the cells were observed with an inverted microscope (TMS-F; Nikon, Tokyo, Japan). Cell survival was evaluated by assaying total proteins by the method of Shopsis & Mackay (1984) after rinsing the cells twice with 2 ml of 0.9% NaCl. Each test was run in triplicate.

Ultrafiltration

Ultrafiltrable zinc was determined as described by Faure *et al.* (1990). For controls, membranes were washed twice with a buffer composed of 10 g l^{-1} $NaHCO_3$, 20 ml l^{-1} NaCl 0.9%, pH 7.4 (HCl N), while membranes used for assay were washed with the same buffer in which NaCl was replaced by 20 ml l^{-1} of a stock aqueous solution of the studied amino acids, giving a final concentration of 0.8 mM. Assays were conducted with samples of 1 ml containing either 20 μl of NaCl 0.9% (controls) or 20 μl of the stock solution of Tau, Cys, His, Gln or Glycine (Gly). Tests were performed with human serum and with Ty-Alb buffer without glucose (albumin and zinc concentrations in this buffer were lower than in serum, but proportions were the same). Each test was repeated six times.

Assessment of zinc bioavailability

Cells were distributed in Petri dishes and grown to confluence. Cultures were washed three times with 1 ml of phosphate buffered saline (PBS) (Guiraud *et al.* 1992).

RPMI 1640 plus 15% FCS medium was replaced by 2 ml of Ty or Ty-Alb buffer, alone or containing the nutrient mixture diluted 1/5. These solutions were labeled with ^{65}Zn ($^{65}ZnCl_2$ in 0.5 N HCl, NEZ 111, NEN du Pont de Nemours, Bad Homburg, Germany) at a concentration of 0.5 μCi ml^{-1}. The fibroblasts were then incubated for 30, 60 and 120 min. At each point, cell labeling was terminated by eliminating the radioactive medium. Cells were rinsed with four 2 ml aliquots of Puck's Saline A (Gibco) containing 10 mM ethylenediaminetetraacetic acid (EDTA; Sigma, St Louis, MO) (Guiraud *et al.* 1992). After harvesting the cells, radioactivity as gamma emission was determined for 1 min. Results were expressed on the basis of the quantity of proteins assayed in cultures treated in these conditions. The three amino acids, i.e. Gln, His and Tau (Sigma), were added to Ty-Alb buffer, alone or containing BM so as to obtain final concentrations of 4.2, 4.2 and 0.8 mM (level of Tau in pediatric TPN), respectively. The level of Gln contained in RPMI 1640 culture medium was 4.2 mM, this value corresponds an increase of 100% of the level of His in the nutrient mixture. The effect of these amino acids on zinc bioavailability was studied with the same experimental procedure.

Each kinetics of ^{65}Zn uptake was run eight times and the results were compared with an analysis of variance (ANOVA). The significance threshold was set at 0.05.

Results

In preliminary test (results not shown), cells were incubated with BM, pure or diluted 1/2, 1/5, 1/10 and 1/20, for 15, 30, 60 and 120 min. The pure mixture was very toxic because of its high osmolarity, leading to a high rate of cell mortality (as early as several minutes of incubation). The minimal dilution consistent with satisfactory cell survival was 1/5 in Ty buffer.

The results obtained with MRC5 cells are summarized in Table 2. The addition of BM (1/5) increased the zinc concentration of the media, since Ty buffer contained 1.8 ± 0.6 μg per 100 ml of zinc and BM/Ty contained 5.7 ± 1.2 μg per 100 ml. Similarly, Ty-Alb buffer contained 4.5 ± 1.3 μg per 100 ml of zinc, but this value increased to 10.3 ± 0.6 μg per 100 ml when BM (1/5) was added. Nevertheless, these concentrations were lower than those of the usual culture medium (RPMI plus 15% FCS). The addition of BM increased the pH a bit over that of the buffers alone, but it remained acceptable since it returned to the normal value of 7.3 in the presence of CO_2 in the incubator. Osmolarity increased, since that of the BM-Ty mixture was twice that of Ty alone. Survival of cells was 61 ± 8.7 in BM/Ty-Alb versus 100% in Ty-Alb.

Table 2. Survival of cells in 1/5 dilutions of the BM in several buffers as compared with culture medium (RPMI plus 15% FCS), zinc concentration (measured by atomic absorption) and pH of the different media

Medium	Zn (μg 100 ml^{-1})	pH	Percent survival (2h)
Ty	1.8 ± 0.6	7.3 ± 0.05	100
Ty-Alb	4.5 ± 1.3	7.3 ± 0.04	100
BM/Ty	5.7 ± 1.2	7.4 ± 0.13	48 ± 9.5
BM/Ty-Alb	10.3 ± 0.6	7.4 ± 0.17	61 ± 8.7
RPMI + 15% FCS	34.2 ± 4.6	7.3 ± 0.02	100

Results are given as means ± SD (n = 3).

The first zinc uptake experiments showed fast cellular zinc accumulation Ty buffer alone, while it was much lower in Ty-Alb buffer, the kinetic curve being closed to that observed when BM was added to the two buffers (Figure 1). Effect of addition of glucose on the flux of nutrients into and out of the cells was investigated by following zinc uptake by cells placed in Ty-Alb containing 1 (basal level), 10, 50 and 100 g l^{-1} of glucose. Osmolarities of these solutions were between 280 and 700 mOsm kg^{-1}, and pH values were between 7.3 and 7.7. Results showed that these variations of physicochemical conditions over short time periods (2 h) did not affect zinc uptake by MRC5 cells (not represented).

A more detailed study of zinc uptake kinetics by cultured fibroblalsts was then carried out in Ty-Alb buffer whose osmolarity and zinc concentration were adjusted to those of the BM (1/5)/Ty-Alb mixture. In parallel, the effect of adding Gln and Tau and that of increasing the His concentration were studied. The kinetics of ^{65}Zn uptake by fibroblasts incubated in the eight media are shown in Figures 2 (30 min incubation), 3 (60 min incubation) and 4 (120 min incubation). Regardless of the incubation time, the presence of BM (1/5) in Ty-Alb buffer significantly enhanced cellular zinc uptake ($P < 0.0001$). The addition of His to Ty-Alb improved zinc uptake, starting at 60 min of incubation, but this increase became significant only after 120 min of incubation. When the His concentration was doubled in BM/Ty-Alb, there was initially (30 min incubation) a significant decrease in cellular zinc uptake, while at 60 and 120 min the results differed little from those obtained with controls in BM/Ty-Alb. Gln slightly increased zinc uptake, regardless of the incubation time and regardless of the medium to which it was added

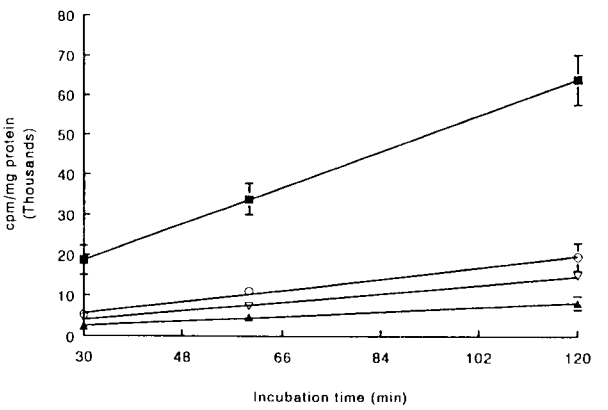

Figure 1. Incorporation of radioactive zinc by MRC5 fibroblasts as a function of incubation time in different media (■, Ty; ○, BM/Ty; ▲, Ty-Alb; ▽, BM/Ty-Alb). Results are given as means ± SD (n = 3).

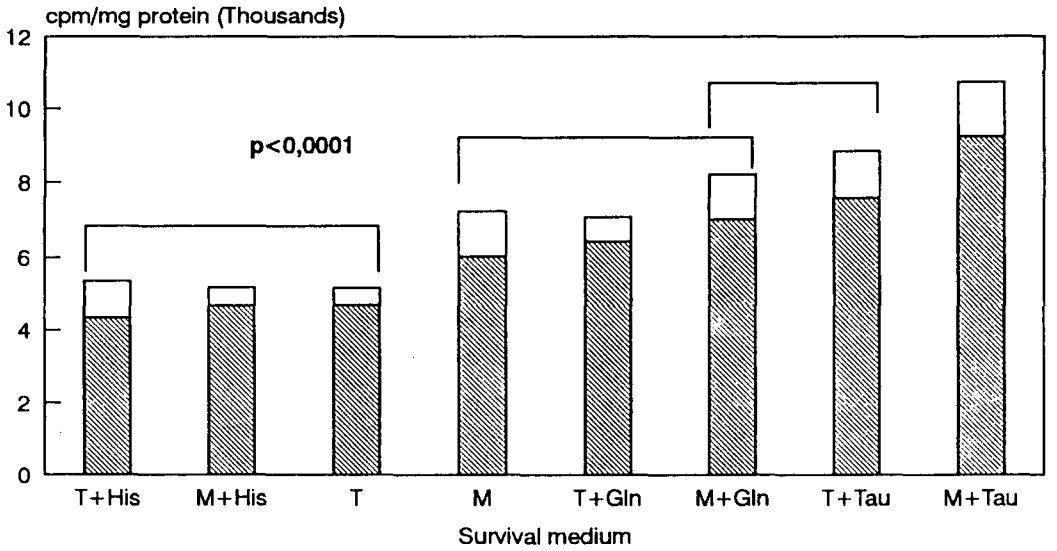

Figure 2. Incorporation of radioactive zinc by MRC5 fibroblasts after 30 min of incubation in the different media tested (T, Ty-Alb; M, BM/Ty-Alb). Histogram bars under the same bracket are not significantly different. Bars under different brackets are significantly different. ▨, mean; □, sd ($n = 8$)

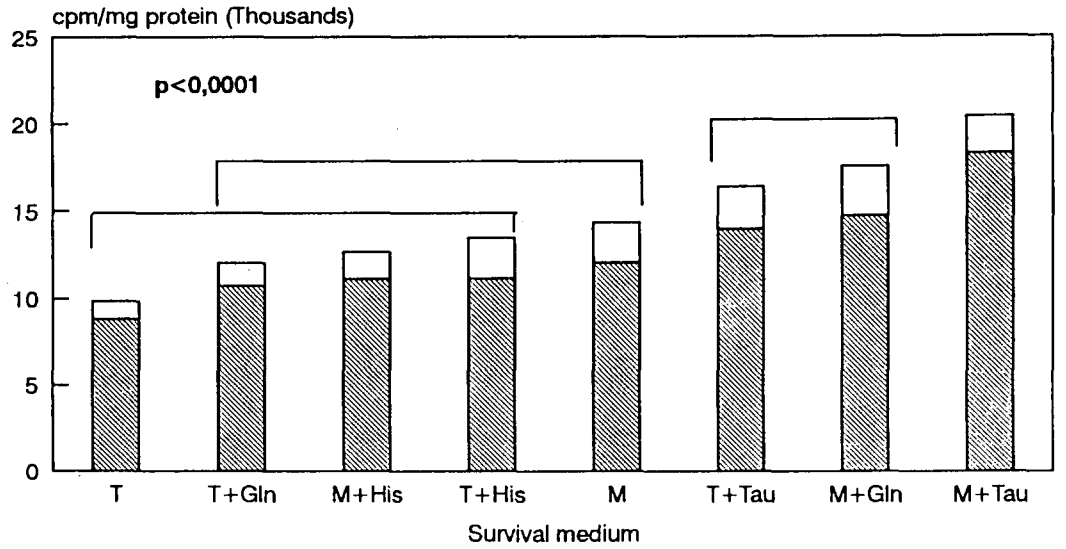

Figure 3. Incorporation of radioactive zinc by MRC5 fibroblasts after 60 min of incubation in the different media tested (T, Ty-Alb; M, BM/Ty-Alb). Histogram bars under the same bracket are not significantly different. Bars under different brackets are significantly different. ▨, mean; □, sd ($n = 8$).

(Ty-Alb: significant increase only at 30 min, or BM/Ty-Alb: significant increase after 60 and 120 min) (Figures 2 and 3). When Tau was added to Ty-Alb, zinc uptake improved significantly as compared with the control, starting at 30 min of incubation. The same phenomenon was observed when Tau was added to BM/Ty-Alb (Figures 2–4). Results of ultrafiltration experiments are given in Table 3. Among the amino acids tested His was the only one able to displace zinc bound to albumin. Its efficiency was higher in Ty-Alb buffer than in human serum. In both cases, results showed that Tau did not react strongly with zinc.

Discussion

The effect of a binary parenteral nutrition mixture on zinc uptake by cultured fibroblasts was initially investigated. The pure mixture was extremely cytotoxic because of its high glucose concentration and thus its elevated osmolarity, explaining why the mixture was diluted. In addition, its

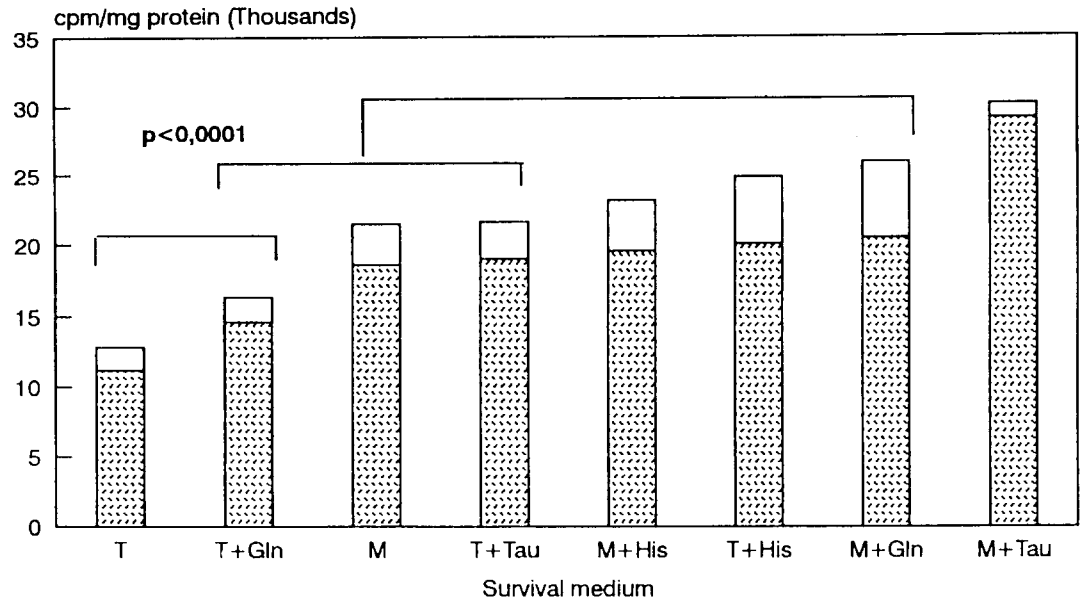

Figure 4. Incorporation of radioactive zinc by MRC5 fibroblasts after 120 min of incubation in the different media tested (T, Ty-Alb; M, BM/Ty-Alb). Histogram bars under the same bracket are not significantly different. Bars under different brackets are significantly different. ⊡, mean; □, sd (n = 8).

Table 3. Evaluation of the zinc binding capability of His, Gln, Cys, Gly and Tau (0.8 mM) in human serum and Ty-Alb buffer without glucose

Human serum	Zinc recovery (%)	Ty-Alb	Zinc recovery (%)
Before UF	100	before UF	100
After UF	4	after UF	57
+ His	22	+ His	100
+ Cys	14	+ Cys	63
+ Gln	6	+ Gln	67
+ Tau	5	+ Tau	67
+ Gly	5	+ Gly	63

UF, ultrafiltration: n = 6.

dilution in use obeys a physiological logic: in the course of perfusion, the nutrient mixture is diluted in the blood plasma. Cytotoxicity tests of 1/5 dilutions of the BM in Ty and Ty-Alb buffers were carried out. As compared with prior experiments, BM reduced cell survival, since it was only 48 ± 9.5% for MRC5 cells after 2 h of incubation in BM/Ty, while it was 87% after 4 h for skin fibroblasts in Ty alone (Guiraud *et al.* 1992). Results obtained with BM/Ty-Alb were similar. Moreover, as in the case of skin fibroblasts, cell survival was higher when Ty-Alb was used in place of Ty.

McArdle *et al.* (1988) described the effect of adding proteins on cellular uptake of trace elements: the addition of albumin to an inorganic buffer containing copper decreased metal uptake by murine fibroblasts. Our results showed that the same was true for zinc, which is in agreement with the fact that albumin is postulated to be the principal zinc vector (zinc exchangeable) in the serum, another part of zinc being ultrafiltrable and probably chelated by amino acids (Scott & Bradwell 1983, Gardiner *et al.* 1984, Foote & Delves 1984, Faure *et al.* 1991). In fact, Ty allowed uptake of free zinc (Zn^{2+}) as there was no ligand in the extracellular medium, while Ty-Alb was nearest to physiological conditions. Ty-Alb was thus prefered as the dilution buffer to study the influence of amino acids.

The effect of amino acids on the cellular bioavailability of zinc appears to differ as a function of cell type. Thus, His activates zinc uptake in intestinal cell vesicles (Seal & Heaton 1983), while it reduces zinc uptake by fetal fibroblasts (Statter & Krieger 1983). Our results showed that the addition of an amino acid/glucose solution to Ty buffer alone considerably decreased zinc uptake by fibroblasts. When BM was diluted in Ty-Alb, cellular zinc uptake was significantly higher than that obtained with Ty-Alb alone. This can be explained by the considerable supply of amino acids resulting from the addition of 20% BM; in particular His, Cys and arginine, amino acids known for their ability to bind zinc. The metal is probably displaced from albumin by certain amino acids, which increases its bioavailability. Our results showed that the addition of His, Gln and Tau to Ty-Alb had a relatively favorable effect on cell bioavailability of zinc. This effect was significant only at 120 min for His, only at 30 min for Gln and from 30 to 120 min for Tau. Competition between albumin and amino acids for zinc has been shown in the serum (Giroux & Henkin 1972, Faure 1978) and also exists at the cellular level.

When the His concentration in BM was doubled, cellular zinc uptake was significantly inhibited after 30 min of incubation. Aiken *et al.* (1992) showed that the effect of His on zinc uptake by rat erythrocytes was a biphasic phenomenon: uptake was facilitated up to an optimal

concentration and then was inhibited. Similarly, an optimal concentration probably also exists for fibroblasts. The amino acid composition of BM studied in this work is close to that of most mixtures currently on the market. They do not contain Tau, except those for pediatric usage. However, it is known that Tau levels are lowered in adults on parenteral nutrition (McArdle *et al.* 1988, Kopple *et al.* 1990) and even though this molecule is a non-essential amino acid, it plays a non-negligible physiological role in the retina and the brain, and also has an effect of the proliferation of cells in culture (Gaull 1986). Experiments involving the addition of Tau to BM at concentrations used in pediatric nutrient mixtures led to a constant and significant improvement of zinc absorption by fibroblasts, starting at 30 min of incubation. Owing to the structure of the molecule of Tau, the formation of a chelate between zinc and Tau is highly unprobable. According to our results of ultrafiltration experiments, this amino acid can favor zinc penetration in cells, but not via a complexation effect. Moreover, it is known that Tau acts on Ca^{2+} metabolism; however, the possibility of direct complexation of Tau and Ca^{2+} has been refuted by ^{13}C-NMR studies (Irving *et al.* 1982). The action of Tau on Ca^{2+} movements thus appears to be indirect and it is perhaps the same mechanism in the case of Zn^{2+}. Studies have shown that some cells in the organism (brain, retina, heart, liver, platelets and lymphocytes) possess an active transport system for Tau and can accumulate it (Sturman & Gaull 1975, Philipps *et al.* 1978, Huxtable *et al.* 1980, Fukuda *et al.* 1984). Moreover, Tau is recognized as an osmoregulator acting physiologically in different tissues, allowing a variety of cells to preserve constant cell size in face of variations in osmolarity of the medium (Hoffmann & Hendil 1976, Van Gelder 1989). This phenomenon seems to be a general feature and may be involved when cells naturally in conditions of controlled osmolarity are accidentally submitted to hyperosmolar conditions (Sanchez-Olea & Pasantes-Morales 1992). In our experiments, cells were in hyperosmolar conditions. Our results showed that in the absence of Tau these conditions did not act on zinc uptake by cells. However, it is possible that an increased uptake of Tau led to an increase in zinc uptake. This point needs to be further investigated. There is little data available on the interactions between zinc and Tau, although deficiencies of these two substances have been implicated in retinal disorders (Vinton *et al.* 1990) and in epilepsy (Barbeau & Donaldson 1974). Work aimed at more precisely elucidating zinc–Tau relationships is in progress. Gln is usually not added to parenteral nutrition mixtures because of its instability. It was shown by Khan *et al.* (1991), however, that it is stable for 14 days at 4 °C and that its rate of degradation in solution at 24 °C varies only between 0.6 and 0.9% per day. Gln accounts for 60% of the total muscle pool of free amino acids and Fürst *et al.* (1990) showed that its concentration is considerably reduced in the muscle tissue of patients on parenteral nutrition. Its addition to BM at the concentration used for the normal culture of fibroblasts causes a slight increase in zinc uptake.

In conclusion, the addition of binary mixture for parenteral nutrition to a mineral buffer containing zinc bound to albumin increases the cell bioavailability of the metal. Not only does the addition of Tau and Gln, two metabolically important amino acids, have an effect on cellular zinc uptake, but the process is actually considerably favored by Tau. The problem of the long-term stability of mixtures containing Tau and Gln may be posed, however, as well as possible interactions with lipids or other trace elements. Since our results may have direct clinical relevance in terms of zinc metabolism, particularly in patients receiving TPN, it should be interesting to undertake studies *in vivo* to assess the role of Tau for zinc assimilation.

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