cysteine-histidine) are electrically charged and are hence filtrable by the kidney, which can explain the TPN-induced extra-urinary excretion of zinc. The main complexes of copper (copper(histidine)$_2$, copper-histidine-threonine, . . .) however are electrically neutral: this could account for the rapid decrease of the copper plasma level during TPN, although large urinary excretions are not observed since the biliary route is favoured in this case [4].

Compensation for the TPN-induced Neutral Extra-losses. A computer-based quantitative approach was simultaneously proposed [19, 20], aimed at the compensation for the metal extra-losses due to TPN. It was based on the principle that the TPN mixture should contain such overall metal concentrations that the corresponding free ones would be identical with those pertaining to normal blood plasma. It was proved valid by the results relative to calcium and magnesium [19], thereby allowing a rough estimation of the doses of zinc and copper to be added to the TPN solution under consideration [19, 20].

Empirical attempts had already been made by clinicians in order to design such compensating doses [12, 4, 5, 7–10], but the specific excretions due to TPN [8] depend on the very composition of the mixtures [15, 17]. Approximate requirements based on average balance figures had thus only been proposed [2, 9–11]. The fact that the use of computer simulations allows us to adjust the metal doses to the specific composition of each mixture is thus of special interest, the more so as this composition often varies during the course of the treatment, depending on the condition of the patient [3].

The present limitations of this approach are (i) the degree of reliability of the equilibrium constants mentioned above, and (ii) the precision of the free metal ion concentrations in normal blood plasma. So far, 10^{-9} M and 10^{-16} *M* have respectively been used for zinc and copper in our calculations [19, 20, 22]. These figures are of the same order of magnitude as those established by Agarwal and Perrin [26], namely 1.6×10^{-9} *M* and 3.1×10^{-16} *M*. More precise values nevertheless would be highly desirable. It is also noteworthy that the ultimate daily doses of zinc (21 mg) and copper (1.6 mg) proposed for the TPN mixture under study do not take into account the possible metal losses due to the gastrointestinal disease itself.

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Biochemical Indices of Metal Toxicity, Exemplified by Studies of Nickel Toxicity in Rats

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Current methods for evaluating the toxicity of metal compounds in experimental animals include measurements of LD50, body and organ weights, assessments of body-burdens, organ-burdens, and toxicokinetics, histopathological and ultrastructural examinations, hematological, immunological, endocrine, reproductive, mutagenic, teratogenic, and carcinogenic tests, neurophysiological and behavioral observations, and, of especial relevance to this Conference, various biochemical techniques. The biochemical indices of metal toxicity that are considered

in this paper include (*a*) quantitative measurements of metallothionein (MT), glutathione (GSH), and related thiol-compounds in tissues and body fluids; (*b*) induction of microsomal heme oxygenase activity in liver, kidney, and other organs; (*c*) determinations of N-acetyl-glucosaminidase (NAG), beta-2 microglobulin, total protein, and amino acid excretions in urine, and (*d*) HPLC-analysis of metal binding to macromolecular ligands in cytosol and in detergent-solubilized organelles from target tissues. Reference values for these biochemical indices are compiled, based upon analyses of tissues and body fluids from untreated rats, and illustrative abnormal results are presented, derived from studies of the acute toxicity of nickel compounds in rats. For examples, metallothionein concentrations are increased 2 to 4 fold, and heme oxygenase activity is increased 3 to 10 fold in kidney and liver of rats killed 17 h after sc injection of $NiCl_2$ (0.5 mmol/kg). Urinary excretions of NAG and total proteins are increased 2 to 4 fold at 48 h after ip injection of $NiCl_2$ (0.1 mmol/kg). Under the same conditions, urinary excretions of certain amino acids (Lys, Leu, Ileu, Val) are increased 15 to 23 fold. Gel-filtration chromatography (HPLC on Toyo-Soda SW2000 and SW3000 columns) of renal cytosol and detergent-solubilized renal microsomes of rats killed 1 h after im injection of $^{63}NiCl_2$ (0.03 mmol/kg) reveal five distinct macromolecular ^{63}Ni-binding components, with molecular weights from 6,000 to 600,000. A rich array of biochemical tests is available to elucidate toxic effects of metals on the kidney, liver, and other organs. Based upon the author's experience with nickel, measurements of metallothionein, heme oxygenase, and metal-binding ligands in tissue homogenates, and assays of N-acetylglucosaminidase, total proteins, and amino acids in body fluids are particularly sensitive, specific, and significant as biochemical indices of metal toxicity.