always a highly significant correlation between the plasma copper increase and the increase in ceruloplasmin, further studies are needed to determine the relative change in concentration of each of the copper-containing components in the physiologic response to disease.

This same increase in plasma copper concentration occurs in a variety of animal models of inflammation. Since treatment of these inflammations with exogenous low-molecular-weight copper complexes produces antiinflammatory effects the use of copper complexes may be viewed as a physiological approach to treatment. Low-molecular-weight copper complexes have been shown to have antiarthritic effects in man.

In addition to being effective antiinflammatory agents copper complexes have been shown to be effective antiulcer, anticonvulsant, anticancer, and antidiabetic agents. This seeming diverse variety of pharmacologic effects are unified with the hypothesis that copper complexes facilitate or promote tissue repair processes involving copper-dependent enzymes and that arthritis, ulcers, seizures, neoplasia, and diabetes are diseases of specific tissues in disrepair. The corollary to this hypothesis is that the loss or reduction of copper-dependent enzyme mediated processes leads to tissue disfunction which may be re-established with copper complex therapy.

Evidence will be presented to show that non-toxic doses of copper complexes have antiinflammatory activity in recognized models of inflammation and that copper complexes of antiinflammatory drugs are more effective than the parent drugs.

Data will also be presented to show that copper complexes have antiulcer activity and that copper complexes of antiinflammatory drugs, which are well known ulcerogens, are also potent antiulcer agents. This observation supports the view that copper complexes of antiinflammatory drugs are less toxic than the parent drugs and suggests that complexation may have a role in ulcerogenesis.

Anticonvulsant activity of copper complexes in two recognized models of seizure will be presented to show that complexes of non-anticonvulsant and convulsant ligands are effective anticonvulsant agents. In addition, data obtained with a copper complex of an antiepileptic drug suggests that the active form of these drugs may be their copper complexes.

Recognition that neoplastic cells have reduced superoxide dismutase (SOD) activity and that copper complexes have SOD-like activity lead to the investigation of copper complexes as anticancer agents. Data will be presented to show that small molecular weight copper complexes inhibit solid Ehrlich tumor growth and increase survival.

Following the observation that SOD inhibited streptozotocin-induced diabetes, small molecular weight copper complexes were studied and found to inhibit streptozotocin-induced diabetes as well. This observation will be presented in support of a possible role for copper complexes in the treatment of diabetes.

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D5

Total Parenteral Nutrition (TPN) as a Cause of Depletion of Trace Metal Ions. Computer-based Interpretation and Treatment

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Clinical Observations

During the past decade, various clinical data have brought out the compelling evidence that total parenteral nutrition (TPN) does induce abnormal losses of essential trace metal ions [1–3].

Among the latter, special attention has been drawn to zinc and copper. Urinary excretion of zinc rises spectacularly as soon as TPN is started, and plasma levels are eventually affected in long-term patients [4–6], notably after the reversal of the catabolic phase [7–9]. For copper, the urinary loss is less significant than that observed for zinc [6, 10], but the plasma level has been reported to fall rapidly and consistently [4].

These extra-losses are more especially injurious to the patient since zinc and copper are among the main trace metals essential to living systems. Zinc deficiency results in various abnormalities such as central nervous system disturbances, skin lesions [7], impaired immunity, growth retardation [9], and impairment of insulin response [9]. Retarded wound healing and tissue repair are also well documented [11], which constitutes an aggravating factor for patients with trauma [6], surgery [1] or burns [12]. The syndromes of copper deficiency are characterized by anemia, leukopenia, neutropenia, with severe bone demineralisation in infants [1].

Compared to enteral nutrition, TPN is known to promote specific extra losses in metals. It is thereby presumed that specific mechanisms inducing metal deficiency are involved [8].

Attempts at an Experimental Interpretation. To interpret these clinical observations is no straightforward task. Various hypotheses have emerged, based on *in vivo* as well as *in vitro* experiments. All of them hinge upon the fact that a small fraction of metal exists in the form of low-molecular-weight (l.m.w.) complexes [13, 14], the extent of which would be raised by TPN infusion. Amino-acids [15, 16] and sugar-amine compounds [12] were characterized as possibly being responsible for the mobilisation of the metal ions from their protein-bound pool. According to a recent study carried out on dogs [17], cysteine would induce a larger urinary excretion of zinc than would histidine, glycine having no effect.

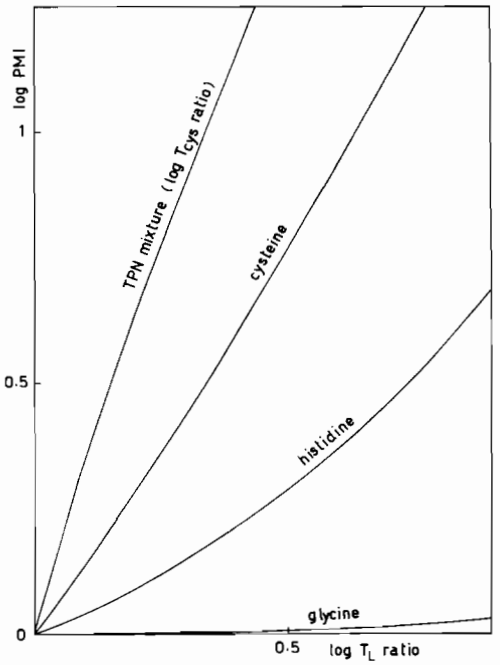
However, no quantitative experimental investigation is possible for l.m.w. complexes in biofluids, due to the extremely low concentrations and to the intricate systems of labile equilibria involved. Computer simulation constitutes the only technique likely to provide a further insight into the problem of the dependence of the metal losses on TPN.

Computer Simulations

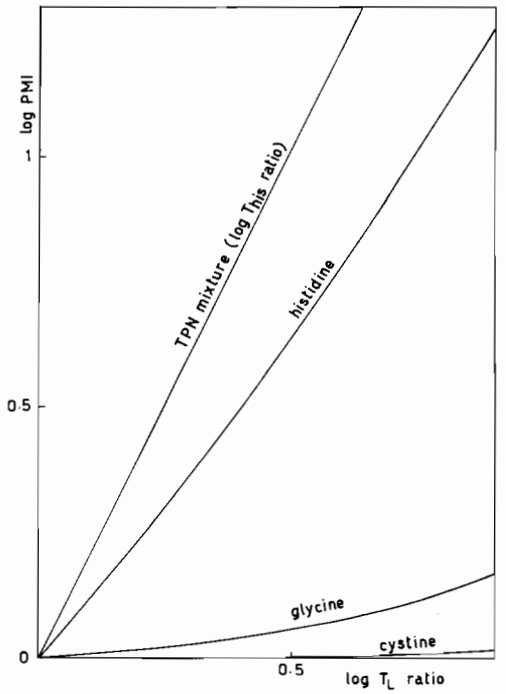
Interpretation of the TPN Effects. In our first work on the topic, the ECCLES programme [18] was used to simulate the metal distribution in the plasma of patients receiving TPN [19, 20]. It appeared that the infusion of the nutritive mixture did result in a significant mobilisation of the metal ions in the form of l.m.w. complexes. Moreover, cysteine and histidine were recognized as the main ligands of zinc, whereas glycine was expected to be inoperative, this being in line with the above observations [17].

Clearly, the reliability of such simulations depends on the equilibrium constants on which the model is based [21]. The constants relative to the main complexes in the nutritive solution were thus determined under the proper experimental conditions, at first for zinc [22], then for copper [23]. The better definition of these constants makes it possible to ascertain the distribution of the related species in blood plasma under TPN* as well as in the TPN mixture itself.

Figure 1 shows the influence of the whole TPN mixture, as compared with that of some of its individual components, on the respective mobilisation of zinc and copper in the form of ultrafilterable l.m.w. complexes. Cysteine and histidine are confirmed as the most mobilising ligands of zinc, histidine being the major ligand of copper. It is also noteworthy that the main complexes of zinc (zinc(cysteine)$_2$, zinc-



(a)



(b)

Fig. 1. PMI curves for the effect of the TPN mixture, of cysteine (or cystine), of histidine and of glycine infusions on the l.m.w. fraction of zinc (a) and copper (b). Log PMI values are plotted against the log of the ratio by which the total concentration of each ligand (T_L) is increased by the infusion. PMI = total concentration of the l.m.w. metal ion fraction in presence of drug/total concentration of the l.m.w. metal ion fraction in normal plasma.

*For example, our discovery [23] of the non-existence of the copper-his-cis and copper-his-cis-H species previously characterized by Hallman *et al.* [24], which was simultaneously confirmed by others [25], is of great importance for the plasma distribution of copper during TPN, account being taken of the significant amounts of histidine (his) and cystine (cis) in the nutritive solution.

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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D6

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