Zinc changes in blood and urine during cyclic parenteral nutrition: relationships with amino acid metabolism

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Serum Zn, ultrafiltrable Zn and amino acids in serum and urine samples of twenty-seven patients receiving cyclic (12 h/24 h) parenteral nutrition were measured. These samples were collected in patients after a 12 h period of parenteral nutrition, and in the evening after 12 h without parenteral nutrition. The same determinations were performed in ten control subjects who followed the same sampling scheme. Total serum ultrafiltrable Zn showed no significant variations in the patients during parenteral nutrition, and was not significantly different in the two groups although the proportion of the Zn present in the ultrafiltrable fraction was elevated. Serum cystine levels were significantly higher ($P = 0{\cdot}05$) in the patients than the control subjects, and cystine excretion was also higher in patients ($P < 0{\cdot}05$) and increased after parenteral nutrition (56·0 (SE 6·5) *v.* 147·1 (SE 20·6) μmol/12 h; $P < 0{\cdot}001$). Histidine levels did not vary significantly in serum after parenteral nutrition and were not different in the patients in comparison with the control subjects. Histidine excretion was not different in the two groups but increased significantly during parenteral nutrition ($P < 0{\cdot}05$). Serum albumin was significantly depressed in the patients compared with the control subjects (45·3 (SE 1·5) *v.* 33·9 (SE 1·5) g/l; $P < 0{\cdot}001$). These results suggest that cystine infusion and excretion relate to the changes occurring in serum Zn and in urinary Zn excretion.

Zinc: Parenteral nutrition: Amino acids

Zinc is an essential trace element which is indispensable for RNA, DNA and protein synthesis. It is a cofactor for at least 200 mammalian enzymes (Riordan, 1976). The metal plays a major role in cell division and metabolism, in growth, immunity and wound healing (Pories *et al.* 1967).

In serum, Zn is transported by numerous components. Serum Zn can be divided into three fractions. One fraction is tightly bound to α2-macroglobulin (Boyett & Sullivan, 1970). This fraction of serum Zn, not available for tissues, represents about 20% of the total serum Zn. A second portion is bound to other proteins, mainly to albumin which carries 70–80% of total serum Zn (Foote & Delves, 1984). Transferrin and immunoglobulins are also thought to carry small amounts of Zn. This Zn is loosely bound, and is in equilibrium with the ultrafiltrable fraction. The last fraction of serum Zn is bound to amino acids. It has been termed diffusible or ultrafiltrable Zn (Faure *et al.* 1990), and only represents 1–3% of total serum Zn. This fraction is thought to play a major role in Zn physiology. It can diffuse within tissues and cells, whereas protein-bound Zn, transported by large molecules (molecular weight > 50000 Da), remains in vessels. However, as this fraction is highly diffusible, it is also involved in Zn excretion, particularly in urinary excretion of Zn.

During the last 18 years many reports have described Zn deficiency in patients receiving

parenteral feeding (Kay & Tasman-Jones, 1975; Arakawa *et al.* 1976; Messing *et al.* 1977; Strobel *et al.* 1978; Takagi *et al.* 1986). Zn deficiency causes skin lesions, similar to those observed in *Acrodermatitis enteropathica*, delayed wound healing, and occasionally stomatitis, glossitis, abdominal pains, diarrhoea and fever.

Amino acids (especially cysteine and histidine), when present in large quantities in blood, bind Zn, removing it from albumin. This then causes excessive Zn excretion in urine with the possibility of subsequent progressive Zn deficiency (Zlotkin, 1989). It has been reported that cysteine is much more effective than histidine or glycine at increasing urinary Zn excretion in dogs (Yunice *et al.* 1978). These urinary losses often aggravate those occurring within the digestive tract (Wolman *et al.* 1979), which are known to be the major cause of Zn deficiency during parenteral nutrition.

The relationship between amino acids, serum albumin, total serum Zn and its subfractions in blood serum and urine does not appear to have been studied in patients undergoing parenteral nutrition, especially when cyclic nutrition is administered. Discontinuous parenteral nutrition is more commonly used and leads to more dramatic changes between fed and fasted periods.

This paper reports the results of a study involving twenty-seven patients receiving partial and discontinuous parenteral feeding.

SUBJECTS AND METHODS

Subjects

This study included twenty-seven patients who received daily parenteral nutrition overnight in our university hospital. There were seventeen males and ten females, aged 18 to 82 years (49·5 (SD 18·2) years). These patients received 4–8 mg supplemental Zn once weekly, and blood sampling and urine collections were undertaken on a day in which they did not receive Zn supplements. The patients had been receiving parenteral nutrition for between 1 and 3 weeks before the study.

The control group consisted of ten healthy laboratory staff. They all volunteered for this study. The sampling scheme for this group was the same as for the patients.

The patients suffered from Crohn's disease (*n* 7), acute pancreatitis (*n* 4), or had undergone gut (*n* 8) or otorhinolaryngologic surgery (*n* 3). One patient suffered from sigmoiditis, one from cirrhosis after chronic alcoholism, one from non-Hodgkin's lymphoma and one from septic shock after a hip prothesis infection.

Patients were enrolled in the study after informed consent was obtained. Patients included in the study were randomly taken amongst those receiving daily parenteral nutrition in the Unit of Parenteral Nutrition of our hospital. Nothing in their treatments was changed by the study. They received 1·5 to 2 litres of parenteral nutrition fluid per night. This provided 200 to 350 g glucose, 64 to 74 g amino acid, 90 to 120 g lipids and 7·54 to 10 MJ (1800 to 2400 kcal). These nutrients covered 75–95% of their requirements. Parenteral nutrition was delivered overnight, between 20.00 hours and 08.00 hours. The Zn content of parenteral mixtures was determined before the study (Chappuis *et al.* 1991) and it was found that they provided between 0·16 and 0·32 mg Zn/d. Zn ingested with food was found to be 2·1 (SD 0·8) mg/d.

The control subjects took their breakfast at 07.30 hours and the patients at 08.30 hours. Lunches were taken at 12.30 hours and dinners between 19.30 and 20.30 hours both by the control and the patient groups. No food was consumed between these meals by either group. Patient and control groups ate bread, butter, jam, coffee or tea, and milk for breakfast. They ate salad, roasted meat or fish, French fries or rice or pasta or vegetables, cheese and one apple or orange for lunch. The dinner consisted of soup, one fried or boiled

egg, vegetables, yogurt and one piece of fruit. Except for coffee, tea and milk, the only drink allowed was fresh water, which was available *ad lib*.

Patients who were given Zn on the day of the study were excluded from the study as were those whose urine collections were incomplete and/or whose serum creatinine was higher than 120 μmol/l.

Sample collection

As parenteral nutrition was delivered only during the night, urine samples were collected from the control group and the patient group between 08.00 and 20.00 hours and between 20.00 and 08.00 hours the night after. The urine sample collected between 08.00 and 20.00 hours was named 'day' urine, and the sample collected between 20.00 and 08.00 hours, 'night' urine. Figures for the whole 24 h were obtained by summing 08.00 to 20.00 hours and 20.00 to 08.00 hours figures. Blood samples were collected at 20.00 and at 08.00 hours.

Methods

Zinc determinations were performed on a Perkin Elmer atomic absorption spectrophotometer, model 560, equipped with a graphite furnace, model HGA 500 (Perkin Elmer, Norwalk, CT, USA). Serum samples were diluted 10-fold before analysis, ultrafiltrable Zn in serum was quantified without dilution after preparation of ultrafiltrates with MPS1 Amicon devices and AN69 Rhone Poulenc membranes (Faure *et al.* 1990). Zn-free plastic ware and tubes were used to collect blood and urine samples and in the analysis. All reagents and deionized water used in these assays also were Zn free.

Amino acid determinations were performed on a 6300 Beckman amino acid autoanalyser calibrated with 250 μmol/l amino acid standards. Cystine results were expressed as 1/2 cystine micromolar concentrations and represent the sum of cystine + cysteine. Cystine is the oxidized form of cysteine and their ratio in blood is roughly constant (cysteine/cystine = 25%; Brigham *et al.* 1960). In physiological fluids, cysteine is spontaneously oxidized to cystine upon standing at room temperature and being exposed to air. This oxidation is catalysed by metal ions (Bremer *et al.* 1981; Malloy *et al.* 1981).

Serum albumin was measured by the bromocresol green method (Doumas *et al.* 1971) and creatinine by Jaffé's picric acid method (Bartels *et al.* 1972) on an Hitachi 717 autoanalyser (Hitachi Ltd, Nataworks, Mito, Japan).

Statistical analysis

Data were analysed with a PCSM Statistical Software (Deltasoft, Meylan, France) running on an 80386 IBM-PC-compatible computer. Series were analysed for normality, and then paired Student's *t* tests were used for intra-group comparisons (day *v.* night in each group), and unpaired Student's *t* tests for inter-group comparisons (patients *v.* control subjects during each period of time). Non-parametric data were tested using the Wilcoxon test or the Mann-Whitney U test. For correlation studies, Pearson's correlation coefficient was calculated for parametric data; it was replaced by a Spearman correlation coefficient for non-parametric data (Sokal & Rolf, 1981).

Differences were considered significant at $P < 0{\cdot}05$.

RESULTS

Results are summarized in Tables 1 (serum samples) and 2 (urine samples).

Weight and creatinine: weight ratio

Although body weights tended to be slightly higher in control subjects than in patients (63·3 (SD 3·26) *v.* 57·4 (SD 4·14) kg, not significant), the urinary creatinine: weight ratios were

significantly higher in control subjects than in patients (0·212 (SD 0·012) *v.* 0·133 (SD 0·009) mmol/d per kg, $P < 0·001$).

Serum zinc

Serum Zn did not change between 20.00 and 08.00 hours in control subjects (Table 1), while in patients a highly significant decrease occurred ($P < 0·0001$) after parenteral nutrition. In patients, compared with control subjects, serum Zn was significantly depressed both at 08.00 hours ($P = 0·005$) and at 20.00 hours ($P < 0·0001$, Fig. 1). In contrast to previous results (Wolman *et al.* 1979; Shulman, 1989), we found no correlation between serum Zn concentration and the time between the last Zn supplement and sampling of blood.

Serum albumin

Serum albumin showed no significant changes in patients and in control subjects between the two samples (20.00 and 08.00 hours). However, patients had much lower concentrations of serum albumin ($P < 0·0001$), compared with control subjects, both at 20.00 hours and at 08.00 hours.

Ultrafiltrable serum zinc

Ultrafiltrable Zn was almost identical in the 20.00 and in the 08.00 hours samples in control subjects, whereas in patients there was a tendency for the ultrafiltrable Zn to decrease, but this was not significant ($P = 0·073$). No significant difference was seen between patients and control subjects (Fig. 1).

Ultrafiltrable Zn is related to total serum Zn (Faure *et al.* 1990); its expression as a percentage of total serum Zn may indicate a physiological change in serum Zn metabolism. This percentage (ultrafiltrable Zn/total serum Zn %) increased significantly in patients at 08.00 hours, while it remained stable in control subjects. These changes resulted in a significantly higher percentage of ultrafiltrable Zn in patients during the day ($P = 0·01$) and at the end of parenteral nutrition ($P < 0·0001$) when compared with control subjects.

Serum amino acids

Results are expressed as total amino acids (Asp + Thr + Ser + Glu + Gln + Gly + Ala + Cys + Val + Met + Ile + Leu + Tyr + Phe + Orn + Lys + His + Arg). There was no significant change in total serum amino acids in either group. However, as the control subjects had a small decrease in total amino acids and the patients a small increase at 08.00 hours, a significantly ($P < 0·05$) higher level of total amino acids was seen in patients at the end of parenteral feeding.

Serum cystine decreased significantly at 08.00 hours in the patients. Inter-group comparisons showed a significant increase in the patients at 20.00 hours ($P = 0·041$), but not at 08.00 hours. Differences in serum histidine concentrations were not significant between the two groups, or between the two periods.

Serum creatinine

Serum creatinine was significantly lower in patients ($P = 0·004$) at the end of parenteral nutrition, and was significantly lower in control subjects than in patients both at 20.00 hours ($P = 0·014$) and 08.00 hours ($P = 0·001$).

Urinary zinc

Urinary Zn (Table 2) did not change in the control group, but increased significantly ($P = 0·003$) in the patients. Moreover, patients excreted significantly higher amounts of Zn than control subjects during the day and during the night ($P < 0·0001$ for both). This

Table 1. *Concentrations of zinc, amino acids, albumin and creatinine in the blood of patients receiving parenteral nutrition between 20.00 and 08.00 hours, compared with healthy controls*‡

(Mean values with their standard errors)

	Control subjects (*n* 10)				Patients (*n* 23)			
	20.00 hours§		08.00 hours§		20.00 hours‖		08.00 hours‖	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Serum zinc (μmol/l)	15·5	1·24	15·2	0·97	11·2††	0·81	8·5***†††	0·70
Ultrafiltrable zinc (μmol/l)	0·429	0·0273	0·415	0·0264	0·422	0·0280	0·368	0·0202
Ultrafiltrable/total serum zinc (%)	2·86	0·212	2·77	0·148	3·98††	0·258	4·72**†††	0·290
Total serum amino acids (μmol/l)	3630	201	3250	143	3760	150	3800†	194
Serum cystine (μmol/l)	33·6	5·29	25·5	4·58	47·4†	4·11	38·7**	3·74
Serum histidine (μmol/l)	104·7	11·22	93·6	19·21	90·1	6·09	94·6	11·13
Serum albumin (g/l)	45·3	1·48	44·0	1·35	33·9†††	1·54	32·0†††	1·03
Serum creatinine (μmol/l)	91	5·0	92	4·3	75†	4·0	67**†††	4·2

Mean values were significantly different from those at 20.00 hours: ** $P < 0·01$, *** $P < 0·001$.
Mean values were significantly different from those of the controls: † $P < 0·05$, †† $P < 0·01$, ††† $P < 0·001$.
‡ For details of subjects and procedures, see pp. 764–765.
§ The 20.00 hours sample was taken before dinner, and the 08.00 hours sample was taken after breakfast.
‖ The 20.00 hours sample was taken before parenteral feeding and the 08.00 hours sample at the end of parenteral feeding.

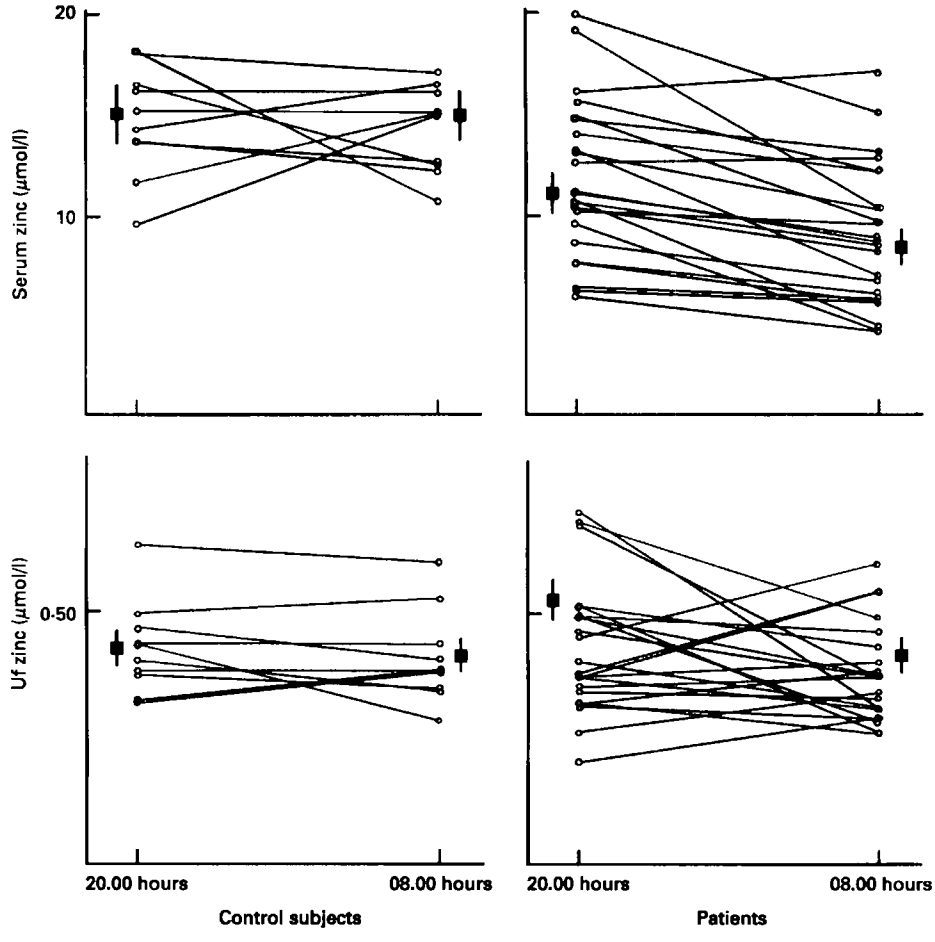

Fig. 1. Variations in total serum zinc and ultrafiltrable (Uf) zinc in patients receiving parenteral nutrition between 20.00 and 08.00 hours, and healthy control subjects. Each pair of points represents a single subject. For details of subjects and procedures, see pp. 764–765.

increased excretion resulted in approximately a 1 mg greater loss of Zn per day in patients than in the control group.

Urinary Zn:creatinine ratio changes showed roughly the same pattern as urinary Zn, but the increase between day and night was not significant in the patients.

Urinary amino acids

Total amino acid excretion showed no significant variation in control subjects, but there was a significant increase ($P = 0{\cdot}008$) during the night in patients.

During the day, amino acid excretion was significantly lower in patients than in control subjects ($P = 0{\cdot}006$). This difference was no longer significant during the night.

The total amino acid:creatinine ratio calculated for the entire 24 h urine collection was significantly increased in patients compared with control subjects ($P = 0{\cdot}03$). The amino acid:creatinine ratio increased significantly during the night in patients. In control subjects this ratio tended to decrease during the night but this was not significant.

Inter-group comparisons showed that urinary cystine excretion was significantly higher

Table 2. *Urinary excretion of zinc, creatinine and amino acids by patients receiving parenteral nutrition for 12 h during the night, compared with healthy controls*‡

(Mean values with their standard errors)

	Control subjects (*n* 10)						Patients (*n* 14)					
	24 h		Day (12 h)		Night (12 h)		24 h		Day (12 h)		Night (12 h)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Urine volume (ml)	1510	232	870	162	630	77·5	1620	147	470	69	1150***††	101
Creatinine (mmol)	13·5	1·04	6·7	0·61	6·8	0·66	7·7†††	0·68	2·7†††	0·22	5·0***††	0·48
Zinc (μmol)	5·6	1·21	2·7	0·63	2·9	0·59	21·2†††	3·19	7·4†††	1·05	14·3***†††	2·39
Zinc/creatinine (μmol/mmol)	0·41	0·069	0·39	0·073	0·43	0·072	2·89†††	0·518	2·76†††	0·425	3·01†††	0·582
Total amino acids (μmol)	4920	594	2610	395	2310	287	5740	1332	1270†	249	4470***	1134
Total amino acids/ creatinine (μmol/mmol)	402	73·9	432	98·5	385	64·4	778†	143·0	463	62·2	946**†	193·3
Cystine (μmol)	57·2	3·63	30·5	3·33	26·7	1·48	203·1†	3·63	56·0†	6·45	147·1***†††	20·60
Cystine/creatinine (μmol/mmol)	4·6	0·54	4·5	0·70	4·3	0·45	27·6†††	2·80	21·4†††	2·80	31·1†††	4·01
Histidine (μmol)	920	127·8	477	76·9	443	56·1	1087	400·6	205†	71·0	882*	337·1
Histidine/creatinine (μmol/mmol)	75	15·1	78	17·5	71	13·2	138	41·1	71	21·2	176*	53·4

Mean values were significantly different from those obtained during the day: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
Mean values were significantly different from those of the controls: † $P < 0·05$, †† $P < 0·01$, ††† $P < 0·001$.
‡ For details of subjects and procedures, see pp. 764–765.

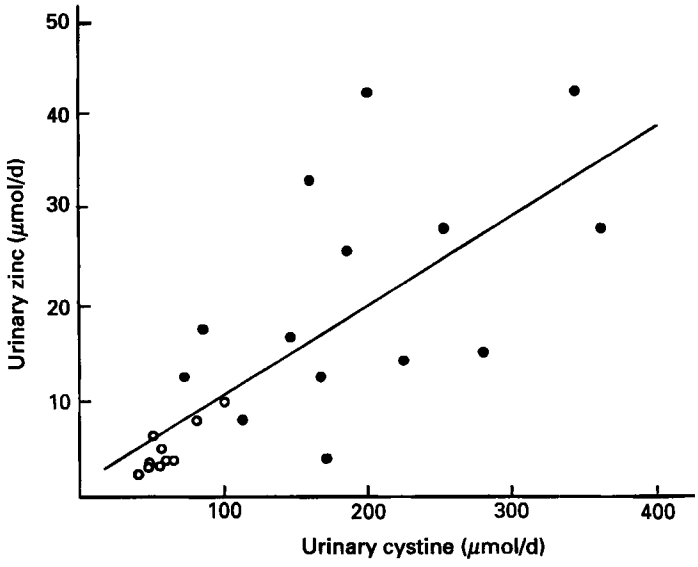

Fig. 2. Relationship between the daily amounts of zinc and cystine excreted in the urine of patients receiving parenteral nutrition between 20.00 and 08.00 hours (●), and healthy control subjects (○). Each point represents a single subject. r 0·762, $P < 0·0001$. For details of subjects and procedures, see pp. 764–765.

in patients both during the day ($P = 0·014$) and during the night ($P < 0·0001$). Histidine excretion in patients was significantly reduced during the day ($P = 0·019$), but was not different during the night. In control subjects cystine excretion remained stable, while it increased significantly in patients during the night ($P = 0·001$).

As shown in Fig. 2, a significant correlation (r 0·76, $P < 0·0001$) existed between urinary Zn and urinary cystine in the entire 24 h urine collection.

DISCUSSION

During parenteral nutrition the negative Zn balances, and Zn-deficiency symptoms that occur in the absence of Zn supplementation, are believed to be the consequence of (a) protein anabolism, which requires Zn, (b) increased digestive losses because these patients often suffer from gut fistula or diseases and (c) increased urinary losses (Freeman *et al.* 1975; Wolman *et al.* 1979). In the absence of Zn supplementation these losses are not replaced by parenteral nutrition mixtures.

The results presented here show a decrease in total serum Zn during infusion of parenteral nutrition mixtures (08.00 hours), in comparison with the period without infusion. This decrease in total serum Zn was accompanied by no change in the ultrafiltrable fraction in serum and an increased urinary excretion of Zn. Serum albumin measurements showed no significant haemodilution in patients between 20.00 and 08.00 hours, but these patients had significantly lower concentrations of the protein than control subjects both at 20.00 and at 08.00 hours. This is the result of malnutrition as confirmed by the low creatinine:weight ratios in patients. Zn has been found to be related to serum albumin (Solomons *et al.* 1976), and its low concentration in our patients also could account (at least partly) for the low serum Zn levels.

It has recently been hypothesized that an increased amino-acid-bound fraction of Zn exists in serum during parenteral nutrition (Cunningham *et al.* 1991). Our results do not

support this hypothesis since we did not find any significant change in serum ultrafiltrable Zn and plasma amino acid concentration. However, ultrafiltrable Zn is correlated with total serum Zn (Faure *et al.* 1990), and a decrease in serum Zn should be accompanied by a decreased ultrafiltrable Zn fraction. When patients are receiving parenteral nutrition, this mechanism cannot operate, since certain amino acids form stable complexes with Zn (Harris & Keen, 1989; Zlotkin, 1989). This leads to an increased proportion of ultrafiltrable Zn which may contribute to enhanced urinary Zn losses.

Blood samples were collected in controls after breakfast (08.00 hours) and in patients at the end of the parenteral nutrition period (20.00 to 08.00 hours) in order to obtain a 'fed' state in both groups. Since food consumption, after a 4 h fasting period, provokes a 9% decrease in serum Zn (Hambidge *et al.* 1989), this effect could interfere with our results. Glucose metabolism was suggested to be responsible for these changes, rather than the changes in plasma amino acids. Hence the parenterally infused glucose also could affect serum Zn values. However, our results show that post-absorptive hypozincaemia alone cannot explain all the serum Zn changes in patients and in control subjects. Moreover glucose is slowly infused over 12 h, and its influence on zincaemia should therefore be smaller than that which occurs following a meal. Concerning amino acids, it should be noted that their infusion is never responsible for transient post-infusion hypoamino-acidaemia (Mosebach *et al.* 1980).

The total amino acid concentration in serum was not significantly increased in patients after parenteral nutrition and was identical to that of control subjects both in the evening and the morning. In our series, only the cystine concentration in serum was higher in patients in the evening (day levels), and, although it decreased after parenteral feeding, it continued to be higher in patients than in control subjects. The percentage of ultrafiltrable Zn in serum followed a similar pattern. Thus it is interesting to note that serum cystine relates more closely to the ultrafiltrable:total Zn ratio than to the absolute figure.

Recent work (Bobilya *et al.* 1993) shows that cysteine and histidine increase the ultrafiltrable Zn fraction in a cell culture medium, and subsequently increase Zn transport into cultured endothelial cells. Hence, a relatively high ultrafiltrable Zn fraction may have two opposite effects: a positive effect by enhancing Zn penetration in cells and a negative one by enhancing Zn losses in urine and in other fluids (e.g. digestive tract, wounds, sweat).

Although patients and control subjects excreted similar amounts of amino acids during the entire 24 h period, patients excreted significantly lower amounts of amino acids during the day. This decreased excretion during the day was probably caused by restriction of water intake during this period compared with that during parenteral feeding. Surprisingly, this reduced excretion of amino acids was not accompanied by a reduced excretion of Zn, but by an increased excretion in patients compared with control subjects. This was probably due to specific increases in plasma cystine and urinary cystine excretion during the day in patients. Our results show that variations in cystine both in urine and plasma can explain much more accurately Zn variations in these fluids than do total amino acids or histidine alone. It has been demonstrated (Yunice *et al.* 1978; Abu Hamdam *et al.* 1981) that cysteine increases urinary Zn excretion by greatly increasing Zn secretion in the proximal tubules of nephrons. Our results are consistent with this (Fig. 2). The mean amount of cysteine infused was 7 mmol/24 h while the amount excreted in urine was 0·203 mmol/24 h. Hence, higher amounts of cystine excreted during the night may come from that which is present in parenteral mixtures. Alternatively, the higher amounts excreted during the day and higher cystine levels in blood could be due to an increased muscle protein turnover in patients and/or from methionine catabolism.

High cystine concentrations rather than total amino acids and, to a lesser extent, low serum albumin may therefore explain the relatively high level of the ultrafiltrable Zn

fraction in plasma and thus are probably important causes of Zn losses during parenteral nutrition.

Higher cystine concentration in blood may also be the consequence of an increased need for glutathione, which is a major antioxidant helping to inhibit free radicals which may form in pathological conditions. It may therefore be deleterious to withdraw cysteine from parenteral mixtures.

Previous studies of Zn supplementation in patients receiving total parenteral nutrition recommend a supplement of 2·5 mg/d in patients having no digestive disease. In the present study patients received only 4-8 mg/week (1 mg/d) because the amount of oral feeding they maintained was thought to compensate for Zn losses and increased needs. However, eight patients (out of twenty-seven) had serum Zn concentrations below 10 μmol/l both at 20.00 and 08.00 hours, but no patient had a serum Zn lower than 5 μmol/l. Among the eight, three patients had a serum Zn concentration between 5·5 and 6·0 μmol/l. Takagi *et al.* (1986) have shown that, in patients receiving total parenteral nutrition, 50% of patients with initial serum Zn concentrations of between 4·6 and 7·7 μmol/l develop Zn deficiency. To prevent all occurrence of deficiency, we think these patients could benefit from a reinforcement of their Zn supplementation. Following Takagi's remarks, we suggest supplementing patients with a serum Zn concentration lower than 7·7 μmol/l with 2·5 mg Zn/d.

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REFERENCES

Abu Hamdam, D. K., Migdal, S. D., Whitehouse, R., Rabbani, P., Prasad, A. S. & MacDonald, F. D. (1981). Renal handling of zinc: effect of cysteine infusion. *American Journal of Physiology* **214**, F487–F494.

Arakawa, T., Tamura, Y. & Igarashi, Y. (1976). Zinc deficiency in two infants during total parenteral nutrition for diarrhea. *American Journal of Clinical Nutrition* **29**, 198–204.

Bartels, H., Böhmer, M. & Heterli, C. (1972). Serum kreatininbestimmung ohne enteiweissen (Serum creatinine determination without protein precipitation). *Clinica Chimica Acta* **37**, 193–197.

Bobilya, D. J., Briske-Anderson, M. & Reeves, P. G. (1993). Ligands influence Zn transport into cultured endothelial cells. *Proceedings of the Society for Experimental Biology and Medicine* **202**, 159–166.

Boyett, J. D. & Sullivan, J. F. (1970). Distribution of protein bound zinc in normal and cirrhotic serum. *Metabolism* **19**, 148–157.

Bremer, H. J., Duran, H. G. & Kamerling, J. P. (1981). *Disturbances of Amino Acid Metabolism: Clinical Chemistry and Diagnosis*, pp 48–49. Baltimore: Urban-Schwartzenberg.

Brigham, M. P., Stein, W. H. & Moore, S. (1960). The concentrations of cysteine and cystine in human blood plasma. *Journal of Clinical Investigation* **39**, 1633–1638.

Chappuis, P., Arnaud, J., Jaudon, M. C., Zawislak, R. & Bellanger, J. (1991). Concentration de Zn, Cu, Se, et Al dans les solutés nutritifs utilisés par voie parentérale (Concentrations of zinc, copper, selenium, and aluminium in parenteral nutritive solutions). *Annales de Biologie Clinique* **49**, 421–427.

Cunningham, J. J., Lydon, M. K., Briggs, S. E. & DeCheke, M. (1991). Zinc and copper status of severely burned children during TPN. *Journal of the American College of Nutrition* **10**, 57–62.

Doumas, B. T., Ard Watson, W. & Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta* **31**, 87–96.

Faure, H., Favier, A., Tripier, M. & Arnaud, J. (1990). Determination of the major zinc fractions in human serum by ultrafiltration. *Biological Trace Element Research* **24**, 25–37.

Foote, J. W. & Delves, H. T. (1984). Distribution of zinc amongst human serum globulins determined by gel filtration-affinity chromatography and atomic absorption spectrophotometry. *Analyst* **109**, 709–711.

Freeman, J. B., Stegink, L. D., Meyer, P. D., Fry, L. K. & DenBesten, L. (1975). Excessive urinary zinc losses during parenteral nutrition. *Journal of Surgical Research* **18**, 463–469.

Hambidge, K. M., Goodall, M. J., Stall, C. & Pritts, J. (1989). Post-prandial and daily changes in plasma zinc. *Journal of Trace Elements and Electrolytes in Health and Diseases* **3**, 55–57.

Harris, W. R. & Keen, C. (1989). Calculations of the distribution of zinc in a computer model of human serum. *Journal of Nutrition* **119**, 1677–1682.

Kay, R. G. & Tasman-Jones, C. (1975). Zinc deficiency and intravenous feeding. *Lancet* **ii**, 605–606.
Malloy, M., Rassin, D. K. & Gaull, G. E. (1981). A method for measurement of free and bound plasma cysteine. *Analytical Biochemistry* **113**, 407–415.
Messing, B., Poitras, P. & Bernier, J. J. (1977). Zinc deficiency in total parenteral nutrition. *Lancet* **ii**, 97–98.
Mosebach, K.-O., Stoeckel, H., Caspari, R., Müller, R., Schulte, J., Lippoldt, R. & Prahl, M. (1980). Pharmacokinetic evaluation of a new maintenance solution for severely injured patients. *Journal of Parenteral and Enteral Nutrition* **4**, 346–350.
Pories, W. L., Henzel, J. H., Rob, C. G. & Strain, W. H. (1967). Acceleration of wound healing in man with zinc sulphate given by mouth. *Lancet* **i**, 121–124.
Riordan, J. F. (1976). Biochemistry of zinc. *Medical Clinics of North America* **60**, 661–674.
Shulman, R. J. (1989). Zinc and copper balance studies in infants receiving total parenteral nutrition. *American Journal of Clinical Nutrition* **49**, 879–883.
Sokal, R. R. & Rohlf, F. J. (1981). Single classification goodness of fit tests. In *Biometry*, pp. 716–721 [J. Wilson and S. Cotter, editors]. New York: W. H. Freeman.
Solomons, N. W., Rosenberg, I. H. & Sandstead, H. (1976). Zinc deficiency in Crohn's disease. *Digestion* **16**, 87–95.
Strobel, C., Byrne, W. J., Abramovitz, W. & Ament, M. E. (1978). A zinc deficiency dermatitis in patients on total parenteral nutrition. *International Journal of Dermatology* **17**, 575–581.
Takagi, Y., Okada, A., Itakura, T. & Kawashima, Y. (1986). Clinical studies on zinc metabolism during total parenteral nutrition as related to zinc deficiency. *Journal of Parenteral and Enteral Nutrition* **10**, 195–202.
Wolman, S. L., Anderson, G. H., Marliss, E. B. & Jeejeebhoy, K. H. (1979). Zinc in total parenteral nutrition: requirements and metabolic effects. *Gastroenterology* **76**, 458–467.
Yunice, A. A., King, R. W., Kraikitpanitch, S., Haygood, C. C. & Lindeman, R. D. (1978). Urinary zinc excretion following infusion of zinc sulfate, cysteine, histidine or glycine. *American Journal of Physiology* **235**, F40–F45.
Zlotkin, S. H. (1989). Nutrient interactions with total parenteral nutrition: effects of histidine and cysteine intake on urinary zinc excretion. *Journal of Pediatrics* **114**, 859–864.

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