SERUM ZINC AND COPPER CONCENTRATIONS IN CHILDREN WITH PROTEIN-CALORIE MALNUTRITION*

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Zinc and copper deficiency in man has not been unequivocally demonstrated, but there is some evidence that a deficiency or disturbance of metabolism of these elements may be of clinical importance.[1-3] Zinc deficiency and dwarfism have been linked,[3,4] although more recently this association has not seemed so clear.[5,6] In protein-calorie malnutrition a reduced copper content of liver and hair has been reported in kwashiorkor.[7] In contrast, the copper content of the liver in marasmus was not found to be reduced.[8] Low serum and plasma zinc in kwashiorkor has been reported in South Africa[9] and Egypt.[10] Plasma copper is also markedly reduced in some cases.[10] Concentrations of both trace elements rose during routine dietary therapy.

Recently the methodology of determining trace element concentrations has been considerably simplified by the introduction of atomic absorption spectrophotometry.[11] A closer study of trace element metabolism in severe nutritional disease has thus become possible with specific relation to possible regional differences. In this communication the concentrations of copper and zinc in the serum of children suffering from kwashiorkor and marasmus before and after therapy are presented. In addition, the zinc output in the urine of 7 cases of kwashiorkor has been observed.

METHODS

Blood samples were taken in the morning, after an 8-hr fast, from 33 cases of kwashiorkor at the time of admission to hospital and before therapy had commenced. In 15 of these a further sample was taken at the time of early recovery (initiation of cure).[12] Blood was also taken from 7 cases of marasmus and 5 cases of marasmic kwashiorkor at the time of admission to hospital and from 15 children who had recovered from burns. These acted as a control group of children who had been on a hospital diet for some weeks. All these children were within the normal Boston percentiles for growth.

The criteria for diagnosis of kwashiorkor, marasmic kwashiorkor and marasmus conform to those laid down by Jelliffe.[13]

All blood samples were taken in disposable syringes with stainless-steel needles and collected in zinc- and copper-free glass test-tubes. Precautions were taken at all times to avoid metal contamination.

Serum samples of 1·0 ml. were used and were analysed in duplicate with a Perkin Elmer Atomic Absorption Spectrophotometer Model 303. The method of preparation of samples was similar to that used by Prasad *et al.*[10] The only modification was that after precipitation with trichloroacetic acid (TCA) the supernatant fluid was chelated with ammonium pyrrolidine dithiocarbamate (APDC) and extracted into methyl-iso-butylketone (MIBK) before being analysed in the flame.

The adequacy of the TCA precipitation method was tested by comparing with the serum zinc and copper analysis of wet-ashed serum samples. Six samples were analysed after ashing with a mixture of nitric, sulphuric and perchloric acids in the ratio of 3 : 1 : 1 and compared to aliquots precipitated with TCA and chelated with APDC. The results showed good agreement between the methods.

*Date received: 28 March 1969.

RESULTS

Serum proteins were determined by the biuret method.[14] In Tables I and II the mean Cu and Zn concentrations in the serum of the various groups of children investigated are presented, together with the serum albumin.

TABLE I. ZINC AND COPPER IN SERA OF CHILDREN WITH PROTEIN-CALORIE MALNUTRITION ON ADMISSION TO HOSPITAL

Clinical material	*No. of cases*	*Zn μg./100 ml. mean±SD (range)*	*Cu μg./100 ml. mean±SD (range)*	*Serum albumin G/100 ml. mean±SD (range)*
Kwashiorkor	33	44±16 (15–81)	61±24 (10–131)	1·81±0·77 (0·72–3·24)
Marasmic kwashiorkor	5	56±9 (46–64)	53±30 (17–95)	2·01±0·63 (1·36–2·85)
Marasmus	7	52±21 (14–87)	93±37 (54–159)	2·69±1·02 (1·71–4·33)
Controls (burns recovery)	15	90±28 (59–163)	140±39 (69–210)	

Zinc

In the control cases the mean serum zinc level was 90 μg./100 ml. In kwashiorkor the mean serum zinc was 44 μg./100 ml. (Table I). In the 15 cases (included in the total series) whose sera were sampled before and after therapy (Table II) the mean serum zinc rose from 42 to 86 μg./100 ml. ($p < 0{\cdot}001$).

In marasmic kwashiorkor and marasmus the mean figures on admission were not significantly different from those in kwashiorkor, being 56 and 52 μg./100 ml. respectively.

The lowest figure obtained was in a case of marasmus (14 μg./100 ml.). This child weighed only 40% of its expected weight for age.

There appears to be a correlation between serum zinc levels and serum albumin concentration on admission ($r = 0{\cdot}71$ $p < 0{\cdot}01$) (Fig. 1).

The urine output of zinc in the 7 cases of kwashiorkor before and after treatment was 286 ± 142 and 342 ± 133 μg./24 hrs respectively, there being no significant differences between the two (Table III).

Copper

In the control cases the mean serum copper level was 140 ± 39 μg./100 ml. (Table I). In kwashiorkor the mean serum copper concentration was 61 μg./100 ml. In the 15 cases studied before and after recovery the figures were 57 and 78 μg./100 ml. respectively ($p < 0{\cdot}1$) (Table II). The admission level in this group is significantly less ($p < 0{\cdot}001$) than the control group. The lowest figure for the

TABLE II. ZINC AND COPPER IN SERA OF CHILDREN WITH KWASHIORKOR BEFORE AND AFTER TREATMENT

				On admission				*On recovery*				
Case No.	*Sex*	*Age*	*Height (cm.)*	*% expt. weight*	*Serum albumin (G/100 ml.)*	*Zn (µg./100 ml.)*	*Cu (µg./100 ml.)*	*% expt. weight*	*Serum albumin (G/100 ml.)*	*Zn (µg./100 ml.)*	*Cu (µg./100 ml.)*	*Time of recovery in days*
1	M	2 yr 8 mth	81	68	1·86	63	72	73	3·43	86	30	12
2	F	1 yr 8 mth	69	55	1·39	30	54	72	4·18	64	31	27
3	M	1 yr 6 mth	73	66	1·28	28	41	68	4·05	104	82	22
4	F	1 yr 5 mth	69	74	0·99	16	60	82	3·99	110	83	30
5	M	2 yr 4 mth	80	87	1·78	42	51	86	3·93	112	175	20
6	M	8 mth	68	85	1·89	35	40	80	3·98	62	41	26
7	M	2 yr 7 mth	76	57	1·36	22	39	61	3·58	80	43	35
8	M	1 yr 5 mth	74	70	3·16	68	44	78	4·56	90	64	9
9	M	2 yr	76	75	1·77	56	131	80	3·48	78	99	21
10	M	3 yr 1 mth	82	60	2·14	40	89	67	4·09	80	94	23
11	F	2 yr 4 mth	77	78	1·45	30	48	83	3·60	83	157	27
12	M	3 yr 5 mth	80	68	1·42	43	51	61	3·53	84	62	18
13	M	1 yr 4 mth	62	47	1·92	46	80	50	4·22	84	46	24
14	M	4 yr 1 mth	83	74	1·42	56	10	66	3·84	89	81	24
15	F	1 yr 6 mth	72	73	1·70	48	32	71	3·83	81	81	21
		Mean		69	1·70	42	57	72	3·86	86	78	23
		± SD		11	0·51	15	21	10	0·32	14	42	7
		Range		47–87	0·99–3·16	16–68	10–131	50–86	3·43–4·56	62–112	30–175	9–35

TABLE III. ZINC LEVELS IN URINE OF CHILDREN WITH KWASHIORKOR BEFORE AND AFTER TREATMENT

Age	*Sex*	*% expt. weight*	*On admission µg. Zn/24 hrs*	*On recovery µg. Zn/24 hrs*	*Time of recovery in days*
2 yr 5 mth	M	63	526	169	37
1 yr 6 mth	M	66	184	247	22
1 yr 8 mth	F	55	208	250	25
2 yr 7 mth	M	61	105	357	35
8 mth	M	85	344	469	25
1 yr 5 mth	M	70	381	355	8
2 yr	M	80	253	550	21
		Mean	286	342	
		SD	±142	±133	
		Range	105–526	169–550	

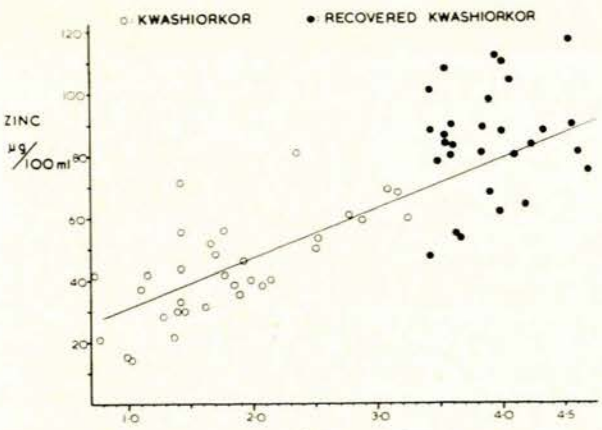

Fig. 1. The relation of serum zinc to serum albumin concentration.

series on admission was 10 µg./100 ml. and the highest 131 µg./100 ml.

In marasmus the serum copper figures were 93 µg./100 ml., which is significantly higher than in kwashiorkor ($p < 0{\cdot}01$) (Table I). In marasmic kwashiorkor the mean figure for serum copper was 53 µg./100 ml. The serum albumin concentration and the serum copper concentration on admission did not appear to be related ($r = 0{\cdot}15$ $p > 0{\cdot}25$) (Fig. 2).

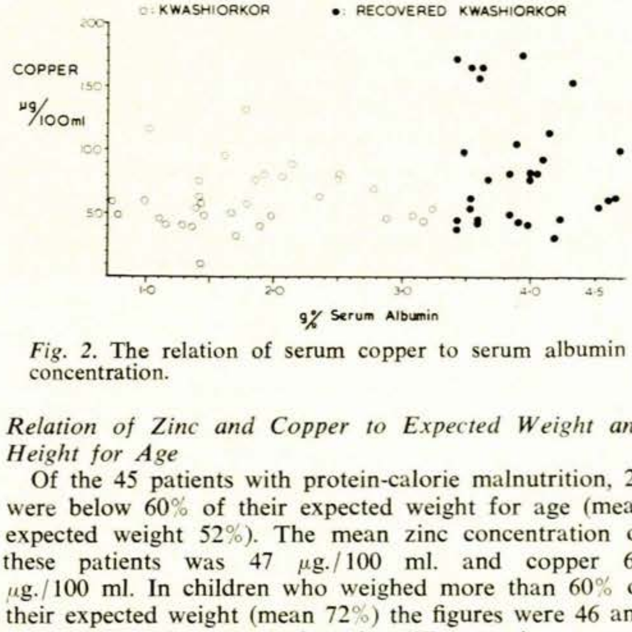

Fig. 2. The relation of serum copper to serum albumin concentration.

Relation of Zinc and Copper to Expected Weight and Height for Age

Of the 45 patients with protein-calorie malnutrition, 22 were below 60% of their expected weight for age (mean expected weight 52%). The mean zinc concentration of these patients was 47 µg./100 ml. and copper 67 µg./100 ml. In children who weighed more than 60% of their expected weight (mean 72%) the figures were 46 and 63 µg./100 ml. respectively. The difference in expected weight between the two groups is significant ($p < 0{\cdot}001$). There is thus no apparent relation of zinc or copper concentration in the serum to expected weight for age in this particular group of patients.

Relation of Zinc and Copper to Clinical Features

No specific relation of zinc and copper levels to severity of the nutritional deficiency or to progress on treatment could be found. There was some association with skin ulceration. Thus, in 19 cases with ulceration of skin, the concentrations were 40 μg./100 ml. for zinc and 51 μg./100 ml. for copper. In 24 cases without ulceration of skin the figures were 50 μg. Zn/100 ml. ($p < 0.05$) and 81 μg. Cu/100 ml. ($p < 0.01$) respectively.

Of 44 patients with protein-calorie malnutrition, 11 had lactose intolerance. The mean zinc concentration of these lactose-intolerant patients was 43 μg./100 ml. and copper 74 μg./100 ml. In children without lactose intolerance the figures were 47 and 69 μg./100 ml. respectively. There was thus no demonstrated relation of zinc and copper serum levels to lactose intolerance.

DISCUSSION

It is apparent from the findings in this series that zinc and copper concentrations in the serum of cases of kwashiorkor and marasmus can be markedly reduced. In some of the individual cases the figures are exceptionally low (less than 20 μg./100 ml. for both elements) compared with those reported in the literature.[1,4,30] With recovery there is a return to levels within—or, in the case of copper, higher than—the reported normal range (76 - 125 μg./100 ml. for zinc[15] and 70 - 140 μg./100 ml. for copper[16]) or our own control cases.

With regard to zinc, our cases appear to have lower levels than have been reported in kwashiorkor elsewhere in this country,[9] but are very similar in general pattern to the cases in Egypt.[10] They are also lower than the concentrations found in growth-retarded older children and young men in Egypt and Iran.[4-6] Comparisons are not always easy, because of different analytical methods used in earlier publications. It has also been reported that plasma zinc concentration is lower than serum zinc concentration.[17] The gross hypo-albuminaemia of kwashiorkor may contribute to the lower level as there seems to be a distinct correlation between zinc and albumin on admission to hospital (Fig. 1). It must also be remembered that zinc concentration is affected by clinical complications such as anaemia and infection.[3,26] The interpretation of serum zinc levels is thus rendered complicated and it is doubtful whether they are a reliable sole indication of zinc deficiency.[7]

Without tracer studies or balance techniques it is difficult to establish the fact of specific zinc deficiency in these malnourished children. Urinary output is not helpful[16,18] and our urinary excretion data bear this out. When compared with published figures for normal children[18] (Fig. 3) the output of zinc in the urine of kwashiorkor patients is somewhat higher. However, reliable excretion data for this age-group could not be found. Our figures are higher than those reported from Pretoria.[9] We have turned to tissue analysis of postmortem material in an attempt to demonstrate zinc deficiency. The initial results[19] do show a decrease of zinc in liver tissue. However, whether this reduction is of physiological importance is not yet clear.

The only correlation we could find of serum zinc levels with specific symptoms or signs was a possible link with ulcerative skin lesions. (This applied also to serum copper concentration.) This is of interest in view of reports that zinc supplements improve the healing of wounds.[20] However, experience shows that the skin lesions of kwashiorkor usually heal very rapidly without zinc or copper supplements. From the retarded growth point of view we were again unable to show any correlation with the degree of dwarfing or being underweight. This is important in view of the great emphasis that has been put on zinc deficiency possibly being related to poor growth.[2-4] In our group of children protein and calorie deficiency *per se* is sufficient to explain growth retardation. It would thus be difficult to attribute it to zinc deficiency, even if we found unequivocal evidence of this.

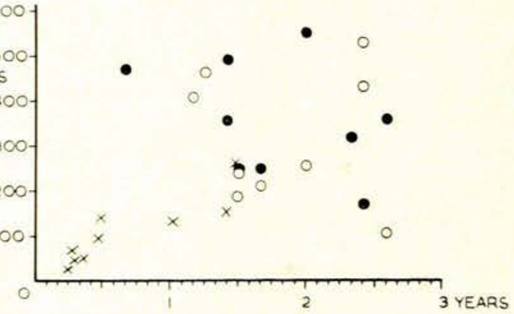

Fig. 3. Urine output of zinc before and after treatment of kwashiorkor.

With regard to copper, the interpretation of the finding of low serum concentrations in the malnourished cases is also difficult. The variation was much greater than in the case of zinc and the rise on recovery was not so dramatic. This is similar to the Egyptian findings.[10] In tissue analysis so far we have confirmed a reduced copper level in the liver in kwashiorkor but not in other tissues.[19] Thus a true over-all copper deficiency has not been demonstrated by our data. This is in line with the statement that copper deficiency has not yet been proved in man.[21] In view of the fact that the copper content of the liver in marasmus is reported to be higher than in kwashiorkor,[8] it is of interest that the serum concentrations in marasmus also tended to be somewhat higher. However, the numbers are small and standard deviation large, so no valid conclusions can be drawn. The reported association between copper deficiency and lactose intolerance[22] was not borne out in our group of patients as judged by serum copper levels and the presence of lactose intolerance. Plasma usually contains 100 μg. copper/100 ml. and about 95% is firmly bound to ceruloplasmin.[23] It is not surprising that the copper concentration could not be linked with serum albumin concentration. Should future work show that there is in fact significantly less copper in the tissues of cases of protein-calorie malnutrition, we are still left wondering whether this has any relation to the clinical state of the patient.

In the meantime the current findings confirm the fact that zinc and copper concentrations in the serum of cases of protein-calorie malnutrition can be very low. The role of trace-element supplementation in the therapy of protein-calorie malnutrition has yet to be explored. Recent reports of the successful use of zinc in wound healing[20] and intestinal malabsorption[24] raise at least interesting possibilities.

SUMMARY

Zinc and copper concentrations of the sera of cases of severe protein-calorie malnutrition have been determined before and after therapy. Both are significantly reduced on admission to hospital and return towards normal values on recovery. The urine output of zinc was not apparently affected in this series. It is not yet established whether low zinc and copper concentrations in the serum can be regarded as evidence of deficiency of these trace elements in protein-calorie malnutrition.

We wish to thank Miss C. Freesemann and Mrs B. MacHutchon for technical assistance. This work was done in the CSIR Clinical Nutrition Research Unit of the University of Cape Town and Red Cross War Memorial Children's Hospital, Rondebosch. Financial assistance was received from US PHS Grant No. AM03995.

REFERENCES

1. Editorial (1965): Nutr. Rev., **23**, 164.
2. Prasad, A. S., Halstead, J. A. and Nadimi, M. (1961): Amer. J. Med., **31**, 532.
3. Prasad, A. S. (1967): Amer. J. Clin. Nutr., **20**, 648.
4. Sandstead, H. H., Prasad, A. S., Schulert, A. R., Farid, Z., Miale, A., Bassilly, S. and Darby, W. J. (1967): *Ibid.*, **20**, 422.
5. Coble, Y. D., Schulert, A. R. and Farid, Z. (1966): *Ibid.*, **18**, 421.
6. Coble, Y. D., Van Reen, R., Schulert, A. R., Koshakji, R. P., Farid, Z. and Davis, J. T. (1966): *Ibid.*, **19**, 415.
7. Macdonald, I. and Warren, P. J. (1961): Brit. J. Nutr., **15**, 593.
8. Godette, L. and Warren, P. J. (1967): *Ibid.*, **21**, 419.
9. Smit, Z. M. and Pretorius, P. J. (1964): J. Trop. Paediat., **9**, 105.
10. Sandstead, H. H., Shukry, A. S., Prasad, A. S., Gabr, M. K., Hifney, A. E., Mokhtar, N. and Darby, W. J. (1965): Amer. J. Clin. Nutr., **17**, 15.
11. Prasad, A. S., Oberleas, D. and Halstead, J. A. (1965): J. Lab. Clin. Med., **66**, 508.
12. Brock, J. F., Hansen, J. D. L., Howe, E. E., Davel, J. G. A., Pretorius, P. J. and Hendrickse, R. G. (1955): Lancet, **2**, 355.
13. Jelliffe, D. B. (1966): *The Assessment of the Nutritional Status of the Community*. Geneva: WHO.
14. Wolfson, W. Q., Cohn, G., Calvary, E. and Ichiba, F. (1948): Amer. J. Clin. Path., **18**, 723.
15. Davies, I. J. T., Musa, M. and Dormandy, T. L. (1968): J. Clin. Path., **21**, 359.
16. Underwood, E. J. (1962): *Trace Elements in Human and Animal Nutrition*. New York: Academic Press.
17. Halstead, J. A., Foley, B., Johnson, S. A., Hackley, B. and Smith, J. C. (1968): Fed. Proc., **27**, 484.
18. Cheek, D. B. (1968): *Human Growth*, p. 427. Philadelphia: Lea & Febiger.
19. Warren, P. J., Hansen, J. D. L. and Lehmann, B. H. (1969): Proc. Nutr. Soc. **28**, (abstr.) 6A.
20. Pories, W. J., Henzel, J. H., Rob, C. G. and Strain, W. H. (1967): Lancet, **1**, 121.
21. Walshe, J. M. (1967): Proc. Nutr. Soc., **27**, 107.
22. Cordano, A. and Graham, G. G. (1966): Pediatrics, **38**, 596.
23. Laurell, C. B. *in* Putnam, F. W., ed. (1960): *The Plasma Proteins*, vol. 1, p. 361. New York: Academic Press.
24. MacMahon, R. A., Le Moine Parker, M. and McKinnon, M. C. (1968): Med. J. Aust., **2**, 210.