# Changes in Trace Elements in Kwashiorkor

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## SUMMARY

**Twenty Black children with classic signs of kwashiorkor were investigated. Serum and hair samples were taken on admission and analysed for several trace elements using emission spectrography and neutron activation analysis. After three weeks on a specific acidified milk diet, serum and hair samples were again taken at the time of discharge and analysed for trace element content.**

**The sera samples were also analysed for total protein, several protein fractions, transferrin and ceruloplasmin levels in an attempt to determine the effect of protein changes. Sera and hair samples of a control group were analysed.**

**It was found that several trace elements are in imbalance in kwashiorkor. The significance of these results is discussed.**

*S. Afr. Med. J.*, **48**, 502 (1974).

Kwashiorkor is a disease of young children and is associated with a deficiency of protein and a relative excess of carbohydrate in the diet. The clinical features of kwashiorkor are by now well known, the main symptoms being: oedema, hyperpigmentation and scaling of the skin, mucous membrane lesions, apathy and irritability, hair changes and retardation of growth.[1]

The main changes in blood composition in kwashiorkor are: (*i*) a low total serum protein concentration (mainly owing to low albumin concentration), and changes in the globulin fractions, the specific changes of which are determined by the pathological stages;[2] (*ii*) a decrease in serum transferrin[3-5] and ceruloplasmin;[6] (*iii*) changes in the plasma aminogram;[7-9] (*iv*) an anaemia responsive to a combination of dietary protein and supplemental iron and/or folic acid.[10-12]

Metabolic changes include those affecting protein,[13,14] carbohydrate[15,16] and lipid[17] metabolism. Hormonal changes are also observed.[18,19]

Evidence exists that trace elements are in imbalance in kwashiorkor. A decrease in the plasma zinc and copper[20] and liver copper[21] has been found. The copper content of hair determined in kwashiorkor patients gave controversial results.[22,23] Blood selenium levels decreased, and the *in vivo* red cell uptake of $^{75}Se$ increased.[23] Chromium imbalance has also been implicated in 4 malnourished patients (1 marasmic and 3 with kwashiorkor); the glucose uptake was severely impaired and the fasting glucose level low. Treatment with chromium chloride resulted in a rapid improvement of glucose uptake and an elevation of the fasting blood glucose level to normal.[24]

Considering the complexity of the disturbances in kwashiorkor, and the functions of the trace elements (especially the role they play in enzyme systems) and the interactions between trace elements, one might expect an imbalance in most or even all of the essential trace elements. It was therefore decided to use multi-element analysing techniques to determine as many of the essential trace elements as possible in the serums of kwashiorkor patients and suitable controls.

In kwashiorkor there is a decrease in serum proteins. Most of the trace elements carried in serum are protein-bound, and therefore changes in the trace element concentration of serum may be influenced by the abnormal serum protein pattern.[17] Total protein was determined and protein electrophoresis done. Specific metal-carrying proteins, such as transferrin and ceruloplasmin were also measured.

The serum level of a trace element does not necessarily reflect other tissue levels and is therefore not indicative of the true body status. In an attempt to obtain more information on the body status of these trace elements, hair samples were analysed. This approach is based on the debatable[25] possibility that hair is important as an indicator of systemic diseases, e.g. malnutrition, which affects the rapidly-dividing cells of the hair root at an early stage.[26,27]

Hair changes are frequently found in children suffering from kwashiorkor.[27] Several studies have been performed on hair of kwashiorkor patients, but only a few attempts were made to relate the changes to trace elements. The only element studied, to our knowledge, was copper, and the results are still inconclusive.[28,29] We therefore decided to determine the concentration of several elements in the hair of kwashiorkor patients and suitable controls.

## PATIENTS AND METHODS

Twenty Black children, with classic signs of kwashiorkor, were investigated. The group consisted of 18 boys and 2 girls, with ages ranging from 11 months to 4 years. The children were put on an acidified milk diet[15] for 3 weeks.

Blood and hair samples were taken on admission. Serum was prepared by the standard procedure. All glassware was washed in 10% nitric acid to avoid contamination with trace elements. The serum was stored at −20°C until analysed. Hair was cut off from the lower part of the head with a clipper and kept in envelopes at room temperature until analysed. After 3 weeks blood and hair samples

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Date received: 23 November 1973.

were again taken. The remaining hair as well as the newly-grown hair, was cut off.

The serum was analysed for the total concentration of several trace elements by emission spectrography according to the method described by Niedermeier *et al.*[29] Total serum protein was estimated according to the Biuret method of Gorwall *et al.*[30] The albumin and various globulin fractions were calculated after electrophoresis on cellulose acetate strips with a Beckman Microzone electrophoresis system. The intensity of the different fractions was read on the Microzone scanning attachment. Serum transferrin and ceruloplasmin levels were determined by immunodiffusion, using Behringwerke M.-Partigen immunodiffusion plates.

The trace element concentrations of the hair samples were determined by applying the technique of neutron activation analysis. Hair samples were washed with a mixture of acetone and ethyl alcohol and dried in an oven at 80°C. Ten-milligram samples, with suitable standards, were irradiated in the Safari-1, an Orr-type reactor of the South African Atomic Energy Board at Pelindaba, for different lengths of time at a neutron flux of $2 \times 10^{13}$ n. $cm^{-2}$. $S^{-1}$. The measurement of the gamma activity of the irradiated samples was commenced after various decay times using a Ge-(Li) detector and a 4 000-channel analyser. A computer programme was used to quantify the elements.

## RESULTS AND DISCUSSION

### Trace Elements in Serum

The results obtained are summarised in Table I. The values obtained for the control groups were surprisingly high compared with reported values, and also higher than the values obtained when the patients were discharged. The values for iron, zinc and copper were confirmed by means of atomic absorption spectrophotometry. Although all the necessary precautions were taken in the quantification of the elements, this study was meant to be comparative. It is of particular importance to compare the values found on admission to the values found at the time of discharge. A statistically significant decrease was found in the iron, zinc, manganese, molybdenum and nickel values of the kwashiorkor patients. The copper value was higher on admission than at the time of discharge, but much lower than the value obtained for the control children. This tendency is confirmed by the ceruloplasmin determinations. It is known that about 90% of the copper in serum is carried by ceruloplasmin.[31,32]

**TABLE I. CONCENTRATION OF TRACE ELEMENTS (PARTS PER MILLION) IN SERUM OF CONTROL CHILDREN AND KWASHIORKOR PATIENTS**

| Element | Control children | Kwashiorkor patients: Admission | Kwashiorkor patients: Discharge |
|---|---|---|---|
| Fe | 2,540 | 0,580* | 1,500 |
| Cu | 2,490 | 0,950* | 1,800 |
| Zn | 2,350 | 0,820* | 1,490 |
| Mn | 0,027 | 0,011* | 0,014 |
| Mo | 0,017 | 0,020 | 0,026 |
| V | 0,083 | 0,050* | 0,064 |
| Ni | 0,081 | 0,036* | 0,052 |
| Co | 0,062 | 0,065 | 0,064 |
| Sr | 0,066 | 0,061 | 0,058 |
| Cr | 0,044 | 0,049 | 0,053 |

* Statistically significant difference from both the control value and the value obtained at the time of discharge ($P<0,05$). Student's *t*-test employed.

### Protein Concentration in Serum

The results of the protein analyses are summarised in Table II. Owing to the fact that the exact concentration of all the elements carried by the serum proteins and the concentration of the specific metalloproteins is not known, a precise recalculation of Table I is not possible. It could, however, be of value to take the data obtained, as well as information from the literature, into consideration for better interpretation of the trace element changes observed.

**TABLE II. CONCENTRATION OF PROTEINS ON SERA OF CONTROL CHILDREN AND KWASHIORKOR PATIENTS**

| | Control children | Kwashiorkor patients: Admission | Kwashiorkor patients: Discharge |
|---|---|---|---|
| Total protein (g/100 ml) | 7,10 | 4,30 | 6,70 |
| Albumin (g/100 ml) | 3,54 | 2,00* | 3,70 |
| $\alpha_1$-globulin (g/100 ml) | 0,31 | 0,22 | 0,20 |
| $\alpha_2$-globulin (g/100 ml) | 1,09 | 0,56* | 0,76 |
| $\beta$-globulin (g/100 ml) | 0,85 | 0,49* | 0,84 |
| $\gamma$-globulin (g/100 ml) | 1,33 | 1,03 | 1,20 |
| Transferrin (mg/100 ml) | 321,7 | 85,0* | 318,4 |
| Ceruloplasmin (mg/100 ml) | 48,8 | 20,6* | 14,6 |

* Statistically significant difference from both the control value and the value obtained at the time of discharge ($P<0,05$). Student's *t*-test employed.

The difference between serum iron values of the kwashiorkor patients on admission and on discharge (Table I), might be explained on the basis of differences in serum transferrin levels (Table II). The fact that the serum transferrin levels of the kwashiorkor patients on discharge is the same as that of the controls, might indicate an undersaturation of the transferrin with iron in the discharged patients.[5] The relative serum copper values reflect to a great extent the relative ceruloplasmin levels in the 3 series of samples. Owing to the fact that both transferrin[33] and ceruloplasmin[34] are not only carrier proteins but also functional proteins, these results implicate functional disturbances in kwashiorkor that might have far-reaching effects.

It is known that about 85% of blood zinc is contained in erythrocytes, 12% in the plasma and the remainder in

the leucocytes.[35] In serum, zinc is bound to albumin and globulin,[36] and especially to $\alpha_2$-globulin.[37] No specific zinc-carrying protein is known. The serum protein results show that both albumin and $\alpha_2$-globulin are decreased in kwashiorkor by about 46% and about 26% respectively. These changes might explain the changes in serum zinc levels.

Manganese is carried by transmanganin, localised electrophoretically[38] on the $\beta_1$-globulin. To date there is no method available for determining this protein. The $\beta_1$-globulin fraction increases in kwashiorkor but this does not suggest a general decrease in manganese concentration.

Vanadium is carried mainly (90%) as free ions[39] in serum, therefore the decrease in serum vanadium might be of primary significance.

In human serum 40% of the nickel is ultrafiltrable and the remainder is approximately equally distributed between albumin-bound nickel and nickeloplasmin (an $\alpha_2$-globulin) nickel.[40] The decrease in serum albumin and $\alpha_2$-globulin partly explains the decrease in serum nickel in kwashiorkor.

From the above data it is clear that knowledge of the concentration of the serum protein fractions might be helpful in explaining the meaning of serum total trace element changes in a disease. Of outstanding importance is the concentration of the specific metalloproteins in serum, because in these complex molecules the protein gives biological specificity to the metal.[41]

The serum protein pattern in kwashiorkor was found to be more or less the same as that shown by other investigators.[2] The transferrin[2-5] and ceruloplasmin[6] levels confirmed the results of previous workers except that in this series the ceruloplasmin was even lower at the time of discharge than at the time of admission. This might have something to do with the diet.

**TABLE III. CONCENTRATION OF TRACE ELEMENTS (PARTS PER MILLION) IN HAIR OF CONTROL CHILDREN AND KWASHIORKOR PATIENTS**

| | Control children | Kwashiorkor patients | | |
|---|---|---|---|---|
| | | | Discharge | |
| | | Admission | Old hair | New hair |
| V | 0,53 | 0,77 | 0,74 | 0,58 |
| Mn | 3,80 | 7,61 | 4,40 | 3,73 |
| Cu* | 39,00 | 30,00 | 30,01 | 45,00 |
| I | 1,00 | 0,92 | 0,72 | 0,87 |
| Ba | — | 4,20 | 3,60 | 3,60 |
| Mo | — | 2,83 | 3,56 | 4,48 |
| Cr | — | 3,20 | 6,40 | 7,40 |
| Se | — | 2,53 | 2,31 | 1,77 |
| Cs | — | 0,37 | 0,46 | 0,40 |
| Cd | — | 16,29 | 6,89 | 11,74 |
| Fe | — | 30,09 | 28,67 | 30,21 |
| Zn | — | 125,50 | 88,07 | 92,93 |
| Co | — | 0,46 | 0,82 | 0,67 |

* The method of neutron activation analysis was used, except for copper which was determined by atomic absorption spectrophotometry.

## Results Obtained from Hair Analysis

This is summarised in Table III. These results are rather disappointing. There appears to be a decrease in the copper and chromium content in the hair of kwashiorkor patients.

Before definite conclusions can be drawn, more results are needed over a period of at least a few months after recovery. More data is also needed of control children in the same socio-economic environment as that of kwashiorkor patients.

REFERENCES

1. Trowell, H. C., Davies, J. N. P. and Dean, R. F. A. (1954): *Kwashiorkor*. London: Edward Arnold.
2. Coward, W. A., Whitehead, R. G. and Coward, D. G. (1972): Brit. J. Nutr., **28**, 433.
3. McFarlane, H., Ogbiede, M. I., Reddy, S., Adcock, K. J., Adeshina, H., Gun, S. M., Cooke, A., Taylor, G. O. and Mordie, J. A. (1969): Lancet, **1**, 392.
4. McFarlane, H., Reddy, S., Adcock, K. J., Adeshina, H., Cooke, A. R. and Akene, J. (1970): Brit. Med. J., **4**, 268.
5. Gabr, M., El-Hawary, M. F. S. and El-Dali, M. (1971): J. Trop. Med. Hyg., **74**, 216.
6. Reiff, B. and Schnieden, H. (1959): Blood, **14**, 967.
7. Whitehead, R. G. (1964): Lancet, **1**, 250.
8. Saunders, S. J., Truswell, A. S., Barbezat, G. O., Wittmann, W. and Hansen, J. D. L. (1967): *Ibid.*, **2**, 975.
9. Prasanna, H. A., Desai, B. L. M. and Ro, M. N. (1971): Brit. J. Nutr., **26**, 71.
10. Halsted, C. H., Sourial, N., Guindi, S., Mourad, K. A. H., Kattab, A. K., Carter, J. P. and Patwardhan, V. N. (1969): Amer. J. Clin. Nutr., **22**, 1371.
11. Adams, E. B. (1969): *Ibid.*, **22**, 1634.
12. Reddy, V. and Srikantia, S. G. (1970): Indian J. Med. Res., **58**, 645.
13. Cravioto, J. (1958): Amer. J. Clin. Nutr., **6**, 495.
14. Edozien, J. C. and Obasi, M. E. (1965): Clin. Sci., **29**, 1.
15. Prinsloo, J. G., De Bruin, E. J. P., Laubscher, N., Venter, L. M. and Kruger, H. (1971): S. Afr. Med. J., **45**, 554.
16. Prinsloo, J. G., De Bruin, E. J. P. and Kruger, H. (1971): Arch. Dis. Childh., **46**, 795.
17. Coward, W. A. and Whitehead, R. G. (1972): Brit. J. Nutr., **27**, 383.
18. Pimstone, B., Becker, D. and Kirnoff, L. (1972): S. Afr. Med. J., **46**, 2102.
19. Kajubi, S. K. (1972): Amer. J. Clin. Nutr., **25**, 1140.
20. Hansen, J. D. L. and Lehmann, B. H. (1969): S. Afr. Med. J., **43**, 1248.
21. MacDonald, I. and Warren, P. J. (1961): Brit. J. Nutr., **15**, 593.
22. Lea, C. M. and Luttrell, V. A. S. (1965): Nature (Lond.), **206**, 413.
23. Burk, R. F., Pearson, W. N., Wood, R. P. and Viteri, F. (1967): Amer. J. Clin. Nutr., **20**, 723.
24. Majaj, A. S. and Hopkins, L. (1966): Leban. Med. J., **19**, 177.
25. Valkovic, V., Miljanic, D., Wheeler, R. M., Liebert, R. B., Zabel, T. and Phillips, G. C. (1973): Nature (Lond.), **243**, 543.
26. Sims, R. T. (1968): Brit. J. Derm., **80**, 337.
27. Crounse, R. G., Bollet, A. J. and Owens, S. (1970): Nature (Lond.), **228**, 465.
28. Trowell, H. C., Davies, J. N. P. and Dean, R. F. A. (1954): *Op. cit.*,[1] p. 80.
29. Niedermeier, W., Griggs, J. H. and Johnson, R. S. (1971): Appl. Spectr., **25**, 53.
30. Gorwall, A. G., Bardawill, C. J. and David, M. M. (1949): J. Biol. Chem., **177**, 751.
31. Holmberg, C. G. and Laurell, C. B. (1947): Acta chem. scand., **1**, 944.
32. *Idem* (1948): *Ibid.*, **2**, 550.
33. Fletcher, J. and Huehns, E. R. (1968): Nature (Lond.), **218**, 1211.
34. Frieden, E. (1970): Nutr. Rev., **28**, 87.
35. Vallee, B. L. (1959): Physiol. Rev., **39**, 443.
36. Surgenor, D. H., Koechlin, B. A. and Strong, L. E. (1949): J. Clin. Invest., **28**, 73.
37. Viesell, E. S. and Bearn, A. G. (1957): Proc. Soc. Exp. Biol. Med., **94**, 96.
38. Cotzias, G. C. and Bertinchamps, A. J. (1961): J. Clin. Invest., **39**, 979.
39. Hopkins, L. L. (Personal communication).
40. Sundermann, F. N., Decsy, M. L. and McNeely, M. D. (1972): Ann. N.Y. Acad. Sci. (Wash.), **199**, 300.
41. Davies, I. J. T. (1972): *The Clinical Significance of the Essential Biological Metals*, p. 72. London: William Heinemann.