Mineral and trace metal supplement for use with synthetic diets based on comminuted chicken

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SUMMARY Earlier studies (Alexander *et al.*, 1974; Lawson *et al.*, 1977) suggested a suitable composition for a mineral and trace metal supplement for use with synthetic diets containing some natural food. Such a mixture has been evaluated in patients receiving a diet based on comminuted chicken and has been shown to be adequate. This conclusion was based on balance experiments measuring Zn, Cu, Mn, Fe, Ca, Mg, N, and P.

A mineral and trace metal mixture (M1) (Aminogran Mineral Mixture, Allen & Hanburys Ltd, or Metabolic Mineral Mixture, Scientific Hospital Supplies Ltd) used with synthetic diets containing some natural foods for the management of inborn errors of metabolism, was found to be inadequate in Zn, Cu, Fe, and Mn (Alexander *et al.*, 1974). An amended mixture (M2) was formulated and contained a 4-fold increase in the content of Zn, a 2-fold increase in Cu, and 25% increase in Fe; Mn was provided separately as tablets (1.1 μ mol; 60 μ g) according to body weight up to a maximum of 6 tablets a day.

Evaluation of M2 in patients with phenylketonuria showed it to be satisfactory for Zn, Cu, and Fe. A minor change was suggested for Mn (Lawson *et al.*, 1977), and this modification was incorporated into a 3rd mixture (M3).

For several years the mineral mixture M1 had been used to supplement a synthetic diet based on comminuted chicken (Cow & Gate Ltd). This is used to treat children with food intolerances and malabsorption for whom milk-, lactose-, and sucrose-free diets are required (Larcher *et al.*, 1977). The patients are often less than one-year old and receive little, if any, natural foods apart from chicken.

Such patients are considered to be at risk from an inadequate intake of minerals and trace metals because of their age, dietary restrictions, and malabsorptive states. A study was designed to evaluate the adequacy of M2 and M3 compared with M1, as supplements to this diet.

The compositions of M1, M2, and M3 are given in Table 1. The results for balances with M1 and M3 are combined as there is no significant difference in their composition.

1.5 g of mixture/kg body weight is added to the feed, up to a maximum of 8 g daily.

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Table 1 Composition of mineral and trace metal supplements (per kg)

	M1	M2	M3
Ca	2.05 mol (82 g)	2.05 (82 g)	2.05 mol (82 g)
K	2.12 mol (83 g)	2.12 mol (83 g)	2.12 mol (83 g)
Na	1.74 mol (40 g)	1.74 mol (40 g)	1.74 mol (40 g)
Mg	399 mmol (9.7 g)	399 mmol (9.7 g)	399 mmol (9.7 g)
Fe	9.00 mmol (550 mg)	11.2 mmol (628 mg)	11.2 mmol (628 mg)
Cu	0.99 mmol (63 mg)	2.00 mmol (127 mg)	2.00 mmol (127 mg)
Zn	1.84 mmol (120 mg)	7.34 mmol (480 mg)	7.34 mmol (480 mg)
Mn	1.04 mmol (57 mg)	—*	0.81 mmol (44.3 mg)
I	60.3 μ mol (7650 μ g)	60.3 μ mol (7650 μ g)	60.3 μ mol (7650 μ g)
Al	10.6 μ mol (285 μ g)	10.6 μ mol (285 μ g)	10.6 μ mol (285 μ g)
Co	17.8 μ mol (1048 μ g)	17.8 μ mol (1048 μ g)	17.8 μ mol (1048 μ g)
Mo	20.7 μ mol (1981 μ g)	20.7 μ mol (1981 μ g)	20.7 μ mol (1981 μ g)
P	1.92 mol (60 g)	1.92 mol (60 g)	1.92 mol (60 g)

*Manganese administered in tablets, each containing 1.1 μ mol (60 μ g).

These mixtures were prepared from simple salts of the metals shown, i.e. chloride, sulphate, and phosphate together with lactate as diluent.

Patients and methods

Metabolic balances were performed on 4 patients aged between 0.16 and 1.53 years who had been on the comminuted chicken diet for at least 3 weeks. Their clinical details are summarised in Table 2. There was a steady weight gain in each, which began before the first balance and was maintained during both studies and in the intervening period. In 3 of them (Cases 1, 3, and 4) the number of stools passed was greater on M2/M3 than on M1, and the other patient (Case 2) passed the same number of stools on both balances.

The diet was based on comminuted chicken as the source of protein with additional fat, carbohydrate, and vitamins as described by Larcher *et al.* (1977). Details of small amounts of additional solids which were milk-, lactose-, sucrose-, and gluten-free, and of medicines, including iron supplements, are shown in Table 3. Additional supplements of Ca (as Ca gluconate) were given to babies receiving less than the recommended 12.1 mmol (500 mg) Ca per day.

Balance studies. Three-day balance studies were performed twice on each child, first on M1 and then on M2, with the exception of Case 4 who

received M3 for his 2nd balance. Case 3 had increased her body weight considerably while at home and received only two-thirds of the correct dose of M2 and manganese tablets as there was no time to equilibrate her on the correct dose before balancing.

Conventional balance techniques were used, with faeces and urine being collected on to deionised nappies (Alexander and Delves, 1972). Analyses of Zn, Cu, Mn, Fe, Ca, Mg, N, and P were performed as described previously (Alexander *et al.*, 1974).

Results

Although urine and faeces were analysed separately there was some contamination between them. Mean daily totals and totals/kg were calculated for the intake, excretion, absorption, and retention of each element on M1 and M2/M3, and the results are shown in Tables 4 and 5. Daily intake was plotted against body weight and regression lines were calculated (Figs 1 to 7, odd numbers). Intake/kg per day was plotted against excretion/kg per day to indicate the net overall balance (Figs 2 to 8, even numbers).

As each child acted as his/her own control, retentions per kg each day on M1 and M2/M3 were compared using a paired *t* test. Percentage absorptions and retentions are shown in Table 6.

Table 2 Details of patients receiving synthetic diets on comminuted chicken

Case	Balance	Sex	Age (years)	Bodyweight (kg)	Weight centile	Period on diet before study (weeks)	Clinical details
1	M1	F	1.42	5.39	<<3	64 M1	Protracted diarrhoea and multiple food intolerances
	M2		1.53	7.40	<3	5 M2	
2	M1	M	0.36	5.60	>3	12 M1	Small bowel resection after necrotising enterocolitis; peritoneal adhesions
	M2		0.53	7.82	>25	8 M2	
3	M1	F	0.29	2.90	<3	4 M1	Protracted diarrhoea; cows' milk and soya protein intolerances
	M2*		0.52	6.60	>10	12 M2	
4	M1	M	0.16	2.85	<<3	3 M1	Protracted diarrhoea; cows' milk protein intolerance
	M3		0.25	3.84	<3	4 M3	

*Received two-thirds of the correct dose of mineral mixture and manganese tablets.

Table 3 Details of diets and medicines

Case	Balance	Iron supplements	Solids in addition to chicken formula	Medicines
1	M1	Yes	RBR, chicken, potato	Sytron†, Ketovite§§ tablets+liquid, metoclopramide
	M2	No	RBR, chicken, potato	Ketovite tablets+liquid, metoclopramide
2	M1	Stopped prebalance	RBR	Ketovite tablets+liquid
	M2	No	RBR, puréed savoury	Ketovite tablets+liquid
3	M1†	Stopped prebalance	None	Ketovite tablets+liquid, metoclopramide, folic acid, nystatin
	M2*	No	None	Ketovite tablets+liquid, folic acid
4	M1†	Yes	None	Ketovite tablets+liquid, ferrous sulphate
	M3	Yes	None	Ketovite tablets+liquid, ferrous sulphate

*Received two-thirds only of the correct dose of mixture and manganese tablets; †received additional calcium gluconate in feeds (see text).

†Sodium iron edetate (Parke Davies & Co.); §§Paines & Byrne Ltd. RBR=Robinson's baby rice.

Table 4 Synthetic diet based on comminuted chicken: metabolic balances. Mean intake and excretion of elements (units/kg per day $\pm$ SD)

Element	Mineral supplement	Intake	Excretion			Absorbed	Retained
			Total	Urine	Faeces		
Zn (μ mol)	M1	5.97 $\pm$ 0.94	7.84 $\pm$ 1.05	1.79 $\pm$ 0.38	6.05 $\pm$ 1.05	-0.08 $\pm$ 1.00	-1.87 $\pm$ 0.34*
	M2/M3	11.56 $\pm$ 3.60	9.23 $\pm$ 3.63	1.58 $\pm$ 0.98	7.65 $\pm$ 2.83	3.91 $\pm$ 2.02	2.33 $\pm$ 1.91
Cu (μ mol)	M1	1.73 $\pm$ 0.35	1.39 $\pm$ 0.33	0.06 $\pm$ 0.02	1.33 $\pm$ 0.31	0.40 $\pm$ 0.50	0.34 $\pm$ 0.51†
	M2/M3	2.23 $\pm$ 0.63	1.72 $\pm$ 0.54	0.13 $\pm$ 0.02	1.59 $\pm$ 0.54	0.64 $\pm$ 0.34	0.51 $\pm$ 0.35†
Mn (μ mol)	M1	1.71 $\pm$ 0.35	1.52 $\pm$ 0.37	0.03 $\pm$ 0.04	1.49 $\pm$ 0.35	0.22 $\pm$ 0.23	0.19 $\pm$ 0.27
	M2/M3	1.51 $\pm$ 0.60	1.24 $\pm$ 0.49	0.04 $\pm$ 0.03	1.20 $\pm$ 0.48	0.31 $\pm$ 0.23	0.27 $\pm$ 0.24
Ca (mmol)	M1	3.43 $\pm$ 0.46	2.78 $\pm$ 1.00	0.12 $\pm$ 0.07	2.66 $\pm$ 0.95	0.77 $\pm$ 0.65	0.65 $\pm$ 0.69
	M2/M3	2.53 $\pm$ 0.62	1.48 $\pm$ 0.34	1.23 $\pm$ 0.03	1.36 $\pm$ 0.32	1.17 $\pm$ 0.46	1.05 $\pm$ 0.48
Mg (mmol)	M1	0.94 $\pm$ 0.08	0.74 $\pm$ 0.12	0.11 $\pm$ 0.07	0.63 $\pm$ 0.06	0.30 $\pm$ 0.02	0.19 $\pm$ 0.07
	M2/M3	0.80 $\pm$ 0.18	0.60 $\pm$ 0.16	0.16 $\pm$ 0.04	0.44 $\pm$ 0.16	0.36 $\pm$ 0.05	0.20 $\pm$ 0.08
N (mmol)	M1	90.40 $\pm$ 10.60	51.50 $\pm$ 8.70	30.10 $\pm$ 10.10	21.40 $\pm$ 10.90	69.00 $\pm$ 10.10	38.90 $\pm$ 2.20
	M2/M3	79.40 $\pm$ 18.30	48.50 $\pm$ 9.10	31.20 $\pm$ 5.60	17.30 $\pm$ 8.30	62.10 $\pm$ 12.20	30.90 $\pm$ 11.30
P (mmol)	M1	3.98 $\pm$ 0.66	2.63 $\pm$ 0.50	1.05 $\pm$ 0.65	1.58 $\pm$ 0.46	2.40 $\pm$ 0.79	1.35 $\pm$ 0.22
	M2/M3	3.78 $\pm$ 0.94	2.27 $\pm$ 0.35	1.33 $\pm$ 0.51	0.94 $\pm$ 0.41	2.84 $\pm$ 0.83	1.51 $\pm$ 0.71

*Differences in retention with M1 compared with M2/M3: P=0.02.

Conversion: SI to traditional units—Zn: 1 μ mol $\approx$ 65.4 μ g; Cu: 1 μ mol $\approx$ 63.5 μ g; Mn: 1 μ mol $\approx$ 54.9 μ g; Ca: 1 mmol $\approx$ 40.1 mg; Mg: 1 mmol $\approx$ 24.3 mg; P: 1 mmol $\approx$ 31 mg; N: 1 mmol $\approx$ 14 mg.Table 5 Mean total intake and retention of elements per day $\pm$ SD

Element	Supplement	Intake		Retention	
		Mean	$\pm$ SD	Mean	$\pm$ SD
Zn (μ mol)	M1	25.5	11.7	-7.4	2.5
	M2/M3	73.3	31.7	16.3	15.4
Cu (μ mol)	M1	7.1	2.1	1.6	1.7
	M2/M3	13.8	4.0	3.4	2.8
Mn (μ mol)	M1	7.5	4.0	1.0	1.2
	M2/M3	10.0	6.0	2.0	1.9
Ca (mmol)	M1	14.2	5.3	3.3	3.5
	M2/M3	15.9	5.4	6.7	3.9
Mg (mmol)	M1	3.9	1.4	0.8	0.5
	M2/M3	5.0	1.4	1.3	0.7
N (mmol)	M1	371	116	160	51
	M2/M3	497	148	196	93
P (mmol)	M1	16.9	7.7	5.8	2.7
	M2/M3	23.7	7.8	9.4	4.9

Table 6 Comparison of percentage absorption and retention of elements on M1 and M2/M3

Element	Supplement	Absorption (%)	Retention (%)
Zn	M1	-1.9	-31.9
	M2/M3	33.3	20.1
Cu	M1	20.8	17.2
	M2/M3	28.4	22.0
Mn	M1	12.8	11.0
	M2/M3	19.7	16.7
Ca	M1	32.2	20.3
	M2/M3	45.2	40.1
Mg	M1	32.4	21.0
	M2/M3	45.8	24.4
N	M1	76.4	43.7
	M2/M3	79.0	37.5
P	M1	59.6	34.1
	M2/M3	75.2	38.0

Discussion

Zinc. The 4 children were in negative Zn balance when receiving M1 (mean retention -31.9%) but in positive balance on M2/M3 (mean retention 20.1%) (Table 6), i.e. retentions of -1.87 μ mol (122 μ g)/kg per day (M1) increasing to +2.33 μ mol (150 μ g)/kg per day (M2/M3). Fig. 1 shows the

Fig. 1 Daily zinc intake v. weight.

Fig. 2 Daily zinc balance.

dietary intake plotted against body weight; the intake increased from a mean of $25.5 \mu\text{mol}$ (1.7 mg)/day on M1 to $73 \mu\text{mol}$ (4.8 mg)/day on M2/M3, equivalent to an increase from $5.97 \mu\text{mol}$ ($390 \mu\text{g}$)/kg per day on M1 to $11.56 \mu\text{mol}$ ($756 \mu\text{g}$)/kg per day on M2/M3. This was accounted for by a 4-fold increase in the Zn content of M2/M3, and in addition the chicken itself provided approximately 60% of the Zn intake from the chicken mixture with M1, and 30% with M2/M3. The differences in intakes on the two mixtures can be seen from the regression lines in Fig. 1. The correlation coefficients for these lines were 0.93 (M1) and 0.65 (M2/M3).

The $73 \mu\text{mol/day}$ that our patients received on M2/M3 was comparable with the 45 to $77 \mu\text{mol}$ (3 to 5 mg) and 57 to $82 \mu\text{mol}$ (3.7 to 5.3 mg) per day recommended by the National Research Council Food and Nutrition Board (1974) and World Health Organisation (1973) respectively. (The latter figures were calculated on the basis of 30% absorption.) Our patients on M1 received a mean of only $25.5 \mu\text{mol}$ per day.

It was concluded that the Zn content of M2/M3 is adequate for use as a supplement with this diet but that M1 provided an inadequate intake.

Copper. For the 4 patients the mean retention increased from $0.34 \mu\text{mol}$ ($21.6 \mu\text{g}$)/kg per day on M1 to $0.51 \mu\text{mol}$ ($32.4 \mu\text{g}$)/kg per day on M2/M3 but the difference was not significant. In Case 4 the balance status changed from -23% retention on M1 to $+16\%$ on M3 and, in Case 1, the increase was from

Fig. 3 Daily copper intake v. weight.

Fig. 4 Daily copper balance.

31% (M1) to 41% (M2) (Table 6). Fig. 3 shows Cu intake plotted against body weight and indicates the increased Cu intake on M2/M3. The correlation coefficients for the regression lines were 0.91 and 0.63 for balances on M1 and M2/M3 respectively.

The mean dietary intake of Cu was 1.73 μmol (110 μg)/kg per day on M1 increasing to 2.23 μmol (142 μg)/kg per day with the higher Cu content of M2/M3 (Table 4). The recommended intake for Cu was estimated at 1.3 μmol (83 μg)/kg per day for infants and young children (World Health Organisation, 1973), and the National Research Council Food and Nutrition Board (1974) suggested between 0.8 to 1.6 μmol (50 to 100 μg)/kg per day so that our children were already receiving more than the recommended intake even on M1 (see Fig. 3). However, Cordano *et al.* (1966) showed that infants with severe marasmus and chronic diarrhoea had depleted Cu stores which could be corrected by Cu supplements of at least 2.36 μmol (150 μg)/kg per day. Furthermore, the children in our study were growing faster than normal and were suffering from malabsorption so one would expect the Cu requirement to be greater than normal.

Toxicity from an excess intake of Cu is unlikely; the Joint Food and Agriculture Organisation/World Health Organisation Food Additives Expert Committee suggested that no deleterious effects in adults should be found up to 8 μmol (0.5 mg)/kg per day (FAO/WHO, 1972). The regulatory mechanism for Cu appears to be excretion in the bile (van Ravesteyn, 1944).

Manganese. All children were in positive Mn balance, except Case 4, while receiving M1 (−12% retention). There was no significant difference between the retentions on M1 compared with M2/M3 (means 0.19 μmol (10.4 μg)/kg per day (11.0%), and 0.27 μmol (14.8 μg)/kg per day (16.7%) respectively (Tables 4 and 6)).

Normal intake of Mn by infants under 6 months was calculated by McLeod and Robinson (1972) to

Fig. 5 Daily manganese intake v. weight.

Fig. 6 Daily manganese balance.

be between 0.05 to 1.37 μmol (2.7 to 75.2 μg)/kg per day, increasing as more solid foods were introduced into the child's diet. World Health Organisation (1973) give the intake of Mn by one- to 3-year-old children as 2.7 to 3.6 μmol (148 to 198 μg)/kg per day. In this present study the mean intake of Mn was 1.7 μmol (93 μg)/kg per day on M1 and 1.5 μmol (82 μg)/kg per day on M2/M3.

Mn was incorporated into the original mineral mixture M1, at an amount equivalent to 1.6 μmol (88 μg)/kg per day for young infants up to a maximum of 8.3 μmol (456 μg) daily (for all children over 5.3 kg). Younger patients in the study by Alexander *et al.* (1974) received an adequate Mn intake on this mixture, but the older patients received significantly less Mn than healthy children of a similar age, probably due to a lower intake of natural foods.

With M2 it was suggested that Mn should be given as one tablet of 1.1 μmol /kg body weight up to a maximum of 6 tablets daily. This alteration in composition results in a slightly lower intake of Mn by these younger children (Fig. 5). However, all the infants in this study appear to be receiving an adequate amount of Mn from M2 and remained in positive balance (Fig. 6).

It is therefore recommended that the formulation of M2/M3 as suggested by Lawson *et al.* (1977) of 6.6 μmol (360 μg) in 8 g mixture is suitable as a supplement for these children.

Iron. M1 and M2/M3 were designed to provide an adequate Fe intake. However, Fe supplements were being given to all the children before the first balance studies (M1) as part of their medical treatment and, in two, it was not considered ethical to withhold these for the purposes of the investigations. This makes the interpretation of our data difficult. Of the 3 balances during which no Fe supplements were given (all on M2) all children showed a positive retention of Fe (29%, 37%, and 8%). The two with the higher retentions were receiving considerable additional Fe from solids. The maximum dose of M2 provides 90 μmol (5 mg) per day and some additional Fe is provided by the chicken. The supplements did not appear to affect absorption of other elements. Patient 4 was on additional Fe during both his 1st and 2nd balances but the changes in absorption and retention of the other elements were similar to those observed in the other children.

Calcium and magnesium. The Ca and Mg content of M2/M3 remained the same as that of M1, since these two elements were found by Alexander *et al.* (1974) to be provided in adequate amounts in the original mixture.

All the children in this study were in positive Mg balance and all except one child were in positive Ca balance (Case 4 on M1 showed -9% retention). As expected, there was no significant difference between the retentions of Ca and Mg when the children were receiving M1 or M2/M3.

All the children studied were offered at least the recommended daily intake of 12.5 mmol Ca, although the actual measured intakes were slightly lower than this in some cases due to rejection of food. The mean intakes of Mg were 3.9 mmol (95 mg) per day (on M1) and 5.00 mmol (122 mg) per day (on M2/M3) (Table 4). World Health Organisation (1973) recommended at least 1.6 to 2.9 mmol (40 to 70 mg) per day for children under one-year old and 4.1 mmol (100 mg) for those between 1 and 2 years.

Nitrogen. During both studies all children received similar nitrogen intakes (Fig. 7) and were in positive nitrogen balance (Fig. 8); there was no significant difference between absorption and retention of N during the 1st and 2nd balances. The mean dietary intake of N (90.4 mmol (1.3 g) and 79.4

Fig. 7 Daily nitrogen intake v. weight.

Fig. 8 Daily nitrogen balance.

mmol (1.19 g)/kg per day on M1 and M2/M3 respectively) is much higher on this chicken-based diet than the intake of healthy children, since a higher percentage of the calorie intake is derived from protein. Retentions of N were 38.9 and 30.9 mmol (0.54 and 0.43 g)/kg per day during the 1st and 2nd balances respectively (Table 4). These high N retentions are probably due to the greater than normal growth velocity, or 'catch-up' growth, which was occurring in the children as they attempted to move towards their ideal weights.

Phosphorus. The P content of M2/M3 remained the same as that of M1. All the children were in positive P balance; mean retentions of 1.35 and 1.51 mmol (41.9 and 46.8 mg)/kg per day were found on M1 and M2/M3 respectively (Table 4), and these were equivalent to percentage retentions of 34.1% (M1) and 38.0% (M2/M3) (Table 6). The mean intakes were 16.9 mmol (524 mg) per day or 3.98 mmol (123 mg)/kg per day on M1 and 23.7 mmol (735 mg) per day or 3.78 mmol (117 mg)/kg per day on M2/M3 (Tables 4 and 5). The National Research Council Food and Nutrition Board (1974) recommend 7.7 mmol (240 mg) per day for children under 6 months old, 12.9 mmol (400 mg) per day between 6 and 12 months, and 25.8 mmol (800 mg) for children over one-year old.

Conclusion

The mineral and trace metal supplement M1 was inadequate for use with this synthetic diet which contained some natural food. Preparations M2/M3 were satisfactory and M3 was more convenient in

use. It should be noted that the comminuted chicken provided about 30% of the total daily intake of zinc when M2/M3 were used. For children on completely synthetic diets without any natural foods, the intake may not be sufficient even from M2/M3, and the alternative supplements suggested by Alexander *et al.* (1974) should be considered.

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References

- Alexander, F. W., Clayton, B. E., and Delves, H. T. (1974). Mineral and trace-metal balances in children receiving normal and synthetic diets. *Quarterly Journal of Medicine*, **43**, 89-111.
- Alexander, F. W., and Delves, H. T. (1972). The preparation of a 'metal-free' nappy and its application to metabolic balances in children. *Journal of Clinical Pathology*, **25**, 927-932.
- Cordano, A., Placko, R. P., and Graham, G. G. (1966). Hypocupremia and neutropenia in copper deficiency. *Blood*, **28**, 280-283.
- Food and Agriculture Organisation/World Health Organisation (1972). *Evaluation of Food Additives. Fifteenth Report of the Joint FAO/WHO Expert Committee on Food Additives*. Technical Report Series No. 488. WHO: Geneva.
- Larcher, V. F., Shepherd, R., Francis, D. E. M., and Harries, J. T. (1977). Protracted diarrhoea in infancy. *Archives of Disease in Childhood*, **52**, 597-605.

- Lawson, M. S., Clayton, B. E., Delves, H. T., and Mitchell, J. D. (1977). Evaluation of a new mineral and trace metal supplement for use with synthetic diets. *Archives of Disease in Childhood*, **52**, 62–67.
- McLeod, B. E., and Robinson, M. F. (1972). Dietary intake of manganese by New Zealand infants during the first 6 months of life. *British Journal of Nutrition*, **27**, 229–232.
- National Research Council Food and Nutrition Board (1974). *Recommended Dietary Allowances*, eighth revised edition. National Academy of Sciences: Washington.
- van Ravesteyn, A. H. (1944). Metabolism of copper in man. *Acta medica Scandinavica*, **118**, 163–196.
- World Health Organisation (1973). *Trace Elements in Human Nutrition*. UN/WHO Technical Report Series 532. WHO: Geneva.

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