Metabolic balance of zinc, copper, cadmium, iron, molybdenum and selenium in young New Zealand women

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1. Metabolic balance studies of zinc, copper, cadmium, iron, molybdenum and selenium were made on four young New Zealand women, using brilliant blue and chromic oxide as faecal markers.

2. Zn, Cu, Cd and Fe concentrations in foods, faeces and urine were measured by atomic absorption spectrophotometry, whereas Mo was determined spectrophotometrically with dithiol and Se fluorimetrically with diaminonaphthalene.

3. The dietary intakes of Zn, Cu and Fe were similar to those reported in the USA and the UK, whereas those of Cd, Mo and Se were less. The subjects ate a diet consisting of foods normally consumed by New Zealand women.

4. For each subject there was little variation in the urinary output of each element for three 6 d periods. Day-to-day variation was small for each subject. The individual variation in urinary output of each element among the subjects was smaller when expressed as a ratio of intake, except for Mo.

5. Retentions were small for Zn, Cu and Fe, all elements which are poorly absorbed. Balances of Se, Mo and possibly Cd were in equilibrium.

Trace-element imbalances have been known to occur in New Zealand in plants and farm animals, but apart from iron, iodine and fluorine little is known about the importance of trace elements in human nutrition in New Zealand. Balance studies were carried out in 1966 and 1967 on four young women (Swindells, Holmes & Robinson, 1968), on a diet consisting of foods normally consumed by a New Zealand woman. Sufficient samples of the diet, urine and faeces remained from these studies to allow the estimation of the trace elements: zinc, copper, cadmium, Fe, manganese, molybdenum and selenium. I and F were not measured as their deficiencies in New Zealand have already been corrected by the use of iodized salt and fluoridated water. Results have been reported previously for nitrogen, lipids, calcium, magnesium and Mn (Swindells *et al.* 1968; Sharpe & Robinson, 1970; McLeod & Robinson, 1972). N and lipids were almost completely absorbed. The balances of Ca and Mg showed the advantage of using simultaneously both intermittent and continuous faecal markers for elements which are not readily absorbed. Small retentions of each element were obtained.

EXPERIMENTAL

Subjects and procedure

The subjects D, W, G and M were students aged 19–21 years and were four of the subjects in a balance study of the metabolic effects of meal frequency (Swindells *et al.* 1968). Each experiment lasted for 27 d; the experimental regimen was divided

into three 6 d experimental periods (2, 3 and 4), with a 6 d preliminary period (1) and a 3 d after-period (5). An amount of food constant for each day was divided into the appropriate number of meals of equal size; three meals were eaten daily in periods 1, 3 and 5, two meals in period 2 (gorging) and nine meals in period 4 (nibbling). The diet consisted of meat loaf and ice-cream with tea and coffee made from the soluble products, and for subjects G and M canned orange juice (Holmes, Swindells, Sharpe, Wright & Robinson, 1969). Each subject chose her own level of fluid intake but kept it constant from day to day, including the volume of tea, coffee and water consumed. Distilled water was used for all beverages but not for washing teeth. The subjects continued to use their customary tooth-pastes. All were habitual non-smokers and had been thoroughly drilled in the procedure required for a reliable balance study.

Brilliant blue was used as an intermittent faecal marker for all subjects (Lutwak & Burton, 1964), and in addition chromic oxide was used as a continuous faecal marker for subjects G and M (Sharpe & Robinson, 1970).

The study with subjects D and W took place in the late spring, November 1966, that of subjects G and M in mid winter, June 1967. Samples of the diet, faeces and urine were collected, stored, and where necessary pooled as described previously (Sharpe & Robinson, 1970; McLeod & Robinson, 1972). Each day six samples of meat loaf were taken from the midday meal, dried in a vacuum oven at 70°, and blended to make the pooled sample for analysis (Swindells *et al.* 1968).

Analytical methods

Fe, Zn, Cu and Cd were determined with a Varian Techtron model AA-5 atomic absorption spectrophotometer (Varian Techtron Pty Ltd, Springvale, Victoria, Australia). Diet, faeces and, when necessary, urine were wet-digested or dry-ashed by the procedures previously described (McLeod & Robinson, 1972). Blanks and standard solutions were included with each set of determinations and treated similarly, as were extra samples with the appropriate trace elements added. Stock urines were usually analysed with each set of urine estimations.

Zn and Fe were determined directly in the acid digests of food and faeces. Zn was determined directly in the undiluted urine, but for Fe, urines were diluted with an equal volume of 60 ml/l butanol in deionized water and read against similarly diluted standard Fe solutions made up in 150 mM-NaCl (Meret & Henkin, 1971). Eighteen replicate analyses of a stock urine over 3 d gave a value of $0{\cdot}159 \pm 0{\cdot}034$ (SD) mg Fe/kg, with a mean recovery of 97%.

For Cu all samples were wet-digested and the Cu present was chelated with ammonium pyrrolidine dithiocarbamate and extracted into methyl isobutyl ketone.

Cd was estimated by the method of standard additions for samples of urine and acid digests of food and faeces (McKenzie, 1972*a*). A typical estimation of 19·4 μg Cd/l urine showed a reproducibility of SD $\pm$ 1·2 μg on 1 d, and of SD $\pm$ 5·2 μg from day to day.

Mo was determined spectrophotometrically with dithiol (toluene-3,4-dithiol),

Table 1. *Composition of foods in the diet*

Food	Year of expt	Concentration/kg dried food: Fe (mg)	Cu (mg)	Zn (mg)	Cd (mg)	Mo (mg)	Se (μg)
Meat loaf	1966	55	3·9	56	0·22	0·09	76
	1967	37	4·8	46	0·08	0·26	59
Ice-cream	1966	11	4·7	27	0·18	0·14	16
	1967	14	4·7	25	0·28	0·15	16
Tea							
Brand A	1966–7	80	7·3	100	0·14	0	40
Brand B	1966–7	76	6·6	77	0·08	0	40
Coffee							
Brand A	1966–7	53	4·0	50	0·36	0·23	160
Brand B	1966–7	85	11·1	22	—	—	150
Orange juice	1967	14	6·6	25	0·20	0·034	150

using the modifications introduced by Healy (1964) for measurement of amounts below 1 μg.

A modification of the diaminonaphthalene fluorimetric method of Watkinson (1966) was used for Se (Robins, 1969). Se could not be determined in faeces containing chromic oxide.

RESULTS

The compositions of the various foods are summarized in Table 1. The items of food were bought in October for the 1966 study and in May for the 1967 study, except for tea and coffee which were bought for both studies in October 1966 and which were from the same batch for each brand. The ice-cream, consisting of evaporated milk, sugar, gelatin and flavouring, varied little in composition between the two studies, whereas the meat loaves made with bread, butter, eggs, meat, potatoes, peas, onions, carrots, tomatoes and seasoning differed in their trace element composition, particularly of Mo and Cd. Differences in composition of the various brands of tea and coffee had little effect upon the daily dietary intake of each element (Table 2). The quantity of ice-cream consumed daily was equivalent to 560 g whole milk and differences in energy needs were made up by the appropriate amounts of meat loaf which were 852 g, 750 g, 675 g, 720 g wet weight for subjects D, W, G and M, respectively (Swindells *et al.* 1968).

Se was determined in fresh supplies of evaporated milk, gelatin and sugar, and from these results the Se concentration of ice-cream was calculated to be 16 μg/kg dry matter. But concentrations of Se in milk and in gelatin may vary by up to ±25% (Grant & Wilson, 1968; Underwood, 1971), to give a range of 1·7–2·6 μg Se for daily intake of Se from ice-cream. Such a range would only alter the total daily intake by up to 5%.

The composition of the dried faeces, pooled according to the use of brilliant blue as faecal marker, is listed in Table 3. The contents of each element or nutrient were similar for each person for the three periods. They were also of the same order for the

Table 2. *Daily intake of trace elements and other nutrients by young women*

(Values in parentheses are considered to be misleading, owing to suspected Fe contamination of the meat loaf)

Subject	Year of expt	Fe (mg)	Cu (mg)	Zn (mg)	Cd (μg)	Mo (μg)	Se (μg)
D	1966	(18·5)	1·85	20·9	92	48	26
W	1966	(16·8)	1·80	19·4	86	46	24
G	1697	12·2	2·01	16·1	60	91	18
M	1967	12·9	2·09	17·0	62	96	19

Table 3. *Weight of dried faeces of young women in periods* 2, 3 *and* 4, *calculated according to brilliant blue (BB) or both chromic oxide and brilliant blue (Cr_2O_3+BB) as faecal markers, and faecal concentration of iron, copper, zinc, cadmium, molybdenum and selenium*

		Wt of dried faeces		Dry matter	Concentration/kg dried faeces					
Subject	Period	BB (g/d)	Cr_2O_3+BB (g/d)	(g/kg wet faeces)	Fe (g)	Cu (g)	Zn (g)	Cd (mg)	Mo (mg)	Se (mg)
D	2	16	—	230	0·65	0·106	0·93	2·4	1·8	0·57
	3	23	—	250	0·67	0·102	1·00	2·4	2·0	0·61
	4	12	—	250	0·76	0·095	1·02	2·6	2·4	0·62
W	2	18	—	310	0·56	0·081	0·80	1·4	1·3	0·43
	3	27	—	280	0·50	0·068	0·72	1·8	1·4	0·45
	4	23	—	240	0·50	0·061	0·67	1·6	1·4	0·42
G	2	19	18	270	0·61	0·089	0·92	2·4	2·5	—
	3	20	18	280	0·59	0·078	0·78	2·4	2·3	—
	4	10	17	290	0·60	0·085	0·98	2·8	2·5	—
M	2	13	19	300	0·66	0·102	0·85	2·6	2·7	—
	3	20	17	330	0·72	0·106	0·94	2·4	2·8	—
	4	6	16	320	0·72	0·101	0·91	2·6	3·1	—

four subjects. According to the intermittent faecal marker brilliant blue or to the continuous faecal marker chromic oxide with brilliant blue, the daily dried weights have been derived for each period, from which the faecal outputs of each element may be deduced (Sharpe & Robinson, 1970). Thus the apparent changes in dried faecal weights with frequency of meals have practically disappeared for subjects G and M, showing an almost constant faecal output of each trace element for the three experimental periods. This had been found previously for N and lipids (Swindells *et al.* 1968) and for Ca and Mg (Sharpe & Robinson, 1970).

The mean values for the faecal losses in the three periods for each subject, expressed as ratios of the intakes, are included in Table 5. Values of about 0·9 of the intake were obtained for Fe, Cu and Zn, whereas those for Cd, Mo and Se, like Mg and Ca, ranged from about 0·4 to 0·7.

In this type of balance study it is impossible to distinguish between the exogenous and endogenous origins of the faecal output. The intestine is the principal route of excretion for many of the trace elements, such as Fe, Cu, Mn and Zn. Thus the faecal

Table 4. *Daily volume of urine excreted by young women in periods* 2, 3 *and* 4 *and daily urinary output of iron, copper, zinc, cadmium, molybdenum and selenium*

(Values are for pooled urines for each period, except for volume and Zn, for which they are means for each period with standard deviations in parentheses)

Subject	Period	Volume (ml)	Fe (mg)	Cu (μg)	Zn (mg)	Cd (μg)	Mo (μg)	Se (μg)
D	2	1791 (87)	—	13	0·34 (0·03)	32	13	16·1
	3	1667 (249)	0·12	11	0·33 (0·05)	54	13	15·3
	4	1811 (191)	0·16	12	0·43 (0·08)	26	17	15·1
W	2	1670 (255)	0·10	36	0·81 (0·23)	16	11	12·6
	3	1382 (146)	0·12	27	0·89 (0·19)	38	14	12·4
	4	1454 (317)	0·10	31	0·71 (0·46)	40	15	11·6
G	2	1026 (84)	0·10	48	0·21 (0·05)	22	51	8·3
	3	1066 (143)	0·12	45	0·25 (0·04)	14	50	7·5
	4	1033 (102)	0·11	41	0·28 (0·07)	28	55	7·7
M	2	1003 (140)	0·11	34	0·24 (0·10)	16	55	11·2
	3	960 (182)	0·12	35	0·24 (0·10)	32	52	11·5
	4	936 (130)	0·07	33	0·26 (0·08)	22	58	10·3

output expressed as a ratio of intake cannot be used as a true measure of the absorption of an element from the dietary intake, but it is a useful rough index.

Table 4 gives the daily urinary output for each period and Table 5 the urinary output expressed as a ratio of intake. For Zn, means and their standard deviations are given for each period in Table 4, but for the other elements the urines had to be pooled to yield sufficient for triplicate estimations of each element, either because its concentration in the urine was so low or because the samples of urine had been almost used up. There is a striking similarity for each subject in the urinary output of each element for the three periods. Thus the frequency of meals did not seem to alter the metabolism of the trace elements as measured by their urinary and faecal output. A measure of the day-to-day variation can only be deduced for Zn; the coefficient of variation (standard deviation expressed as percentage of mean urinary output) varied from 10 to 65 %. This variation is small when it is realized that the daily urinary output represents less than 0·02 of the dietary intake of Zn for subjects D, G and M and 0·04 for subject W (Table 5).

Individual variation in urinary output of each element among the subjects was not

Table 5. *Faecal output (F), calculated according to brilliant blue (BB) or both chromic oxide and brilliant blue (Cr_2O_3+BB) as faecal markers, and urinary output (U) by young women of iron, copper, zinc, cadmium, molybdenum and selenium, expressed as ratios of dietary intake*

(Values in parentheses are considered to be misleading owing to suspected Fe contamination of the meat loaf in the diet)

		Ratio of dietary intake											
		Fe		Cu		Zn		Cd		Mo		Se	
Subject	Faecal marker	F	U	F	U	F	U	F	U	F	U	F	U
D	BB	(0·63)	0·008	0·94	0·006	0·80	0·018	0·45	0·41	0·72	0·31	0·39	0·60
W	BB	(0·70)	0·007	0·87	0·017	0·84	0·043	0·42	0·36	0·68	0·29	0·42	0·50
G	BB	0·84	0·009	0·69	0·022	0·90	0·016	0·69	0·36	0·44	0·57	—	0·43
	Cr_2O_3+BB	0·89		0·73		0·97		0·74		0·47			
M	BB	0·71	0·008	0·65	0·016	0·69	0·014	0·50	0·39	0·38	0·57	—	0·57
	Cr_2O_3+BB	0·95		0·86		0·91		0·73		0·52			

Table 6. *Daily balances or 'unaccounted for amounts' of iron, copper, zinc, cadmium, molybdenum and selenium, expressed as (a) amount/d and (b) ratio of dietary intake, calculated according to brilliant blue (BB) or both chromic oxide and brilliant blue (Cr_2O_3+BB) as faecal markers, in young women*

(Values in parentheses are considered to be misleading owing to suspected Fe contamination of the meat loaf in the diet)

(*a*) Amount/d

Subject	Faecal marker	Fe (mg)	Cu (mg)	Zn (mg)	Cd (μg)	Mo (μg)	Se (μg)
D	BB	(6·7)	0·09	4·0	14	−1·2	0·3
W	BB	(4·9)	0·21	2·3	18	1·4	1·7
G	BB	1·9	0·57	1·4	−2	−0·9	—
	Cr_2O_3+BB	1·3	0·50	0·3	−6	−3·5	—
M	BB	3·7	0·71	5·1	8	4·8	—
	Cr_2O_3+BB	0·5	0·26	1·2	−8	−8·6	—

(*b*) Ratio of dietary intake

Subject	Faecal marker	Fe	Cu	Zn	Cd	Mo	Se
D	BB	(0·36)	0·05	0·19	0·14	−0·03	0·01
W	BB	(0·29)	0·12	0·12	0·22	0·03	0·07
G	BB	0·16	0·28	0·09	−0·04	−0·01	—
	Cr_2O_3+BB	0·10	0·25	0·02	−0·09	−0·04	—
M	BB	0·29	0·34	0·30	0·11	0·05	—
	Cr_2O_3+BB	0·04	0·13	0·07	−0·11	−0·09	—

great and became trivial when the urinary output was expressed as a ratio of the intake (Table 5). For Fe the urinary output:input was less than 0·01, and for Cu and Zn was less than 0·05. The range for Cd was small (0·36–0·41) whereas that for Se was 0·43–0·60. The wider range of 0·30 for Mo in the 1966 study to 0·57 in 1967 resulted from the urinary outputs in the earlier study being one-third of those in the later one.

The difference between the intake and the combined urinary and faecal output gave the amount of each element unaccounted for, and this was taken as the balance. Table 6 lists these values for each element, expressed (*a*) as amount/d, and (*b*) as a ratio of the intake. The high retentions of 6·7 and 4·9 mg Fe for subjects D and W respectively are believed to result from contamination of the samples of meat loaf, which would also give misleading results when the faecal output and the balance are expressed as ratios of the intake. All these values from the 1966 study are given in parentheses in Tables 5 and 6. Contamination of the meat loaf by Cu in the 1967 study is also suspected, but to a much smaller degree.

All elements appeared to be retained by the subjects except Mo, and Cd in subjects G and M. This may have been because dermal loss and menstrual loss were not measured, and no corrections were made for them. For most elements in this study, faeces make the main contribution to the output and, even when the faecal output was corrected according to the recovery of chromic oxide, over 0·1 of the intake was sometimes unaccounted for. The subjects understood the need to minimize the dermal

losses from sweating and desquamation, and were careful not to do anything which might cause sweating. Their surroundings were kept cool, usually below 20°, and this was more easily attained for subjects G and M, who were studied during the winter, than for subjects D and W, who were relaxing after end-of-year examinations in the late spring. There were minor spells of stress at the time the two sets of examination results appeared but these did not coincide with variations in the day-to-day balances of Na or of water (Robinson, unpublished results). Consolazio, Nelson, Matousch, Hughes & Urone (1964) showed that considerable quantities of trace elements were lost under conditions of profuse sweating (7·5 h at 37·8° for 16 consecutive d), accounting for over 0·40 of the intake for Cu, Zn, Se and Mo, but losses of this magnitude were not expected in our study.

Menstruation occurred for subjects D, W, G and M on day 2 period 5, day 1 period 4, day 1 period 3, and day 6 period 1, respectively, and lasted for 4–5 d (Swindells *et al.* 1968). Menstrual losses have been reported of 4–26 mg Fe per menses, 0·5 mg Cu and 0·3–0·6 mg Zn, equivalent to a daily loss of up to 1 mg Fe, 0·02 mg Cu and 0·2 mg Zn (Underwood, 1971). Such losses might account for an output equivalent to 0·01 of the intake for Cu and Zn, and up to 0·07 for Fe.

Even though the intake of Mo in the 1967 study was twice that in the 1966 study for subjects G and M and the outputs in the urine and in the faeces differed in the two studies, the Mo balances were close to being in equilibrium. The Cd balances might also be considered in equilibrium as the apparent balances were within the experimental error of the method for estimating Cd. Se balances from the 1966 study suggest that retention of Se was small or zero. No allowance was made for dermal loss or for respiratory loss.

DISCUSSION

The availability of material from two metabolic balance studies presented a unique opportunity to obtain information about the dietary intake and the corresponding outputs in the faeces and in the urine of several essential trace elements. Moreover the results may be considered along with those for the main nutrients.

Although the diet was in the form of meat loaf, ice-cream, orange juice, and with tea and coffee prepared with distilled water, it contained the kinds and amounts of foods normally consumed by a New Zealand woman, except for green vegetables and raw fruit. Thus, the intake of trace elements by these four women might be expected to give information about the intake of these elements in New Zealand. By comparison with the present recommended intakes of nutrients, the intake was liberal for Zn and adequate for Fe. No special recommendations have been suggested for the other essential trace elements, apart from indicating that they should be included in the diet ((USA) National Research Council: Food and Nutrition Board, 1968; (UK) Department of Health and Social Security, 1969; National Health and Medical Research Council of Australia, 1970). The intake of Cu is among the lower intakes reported in the USA and the UK to be adequate (Schroeder, Nason, Tipton & Balassa, 1966; Walshe, 1968; Schroeder & Nason, 1971). In our study the beverages were made from distilled water. Soft water flowing through galvanized Fe, Cu and plastic pipes takes

up trace elements (Schroeder *et al.* 1966, 1967; Underwood, 1971). The University of Otago (Dunedin) and a large area surrounding it are supplied with soft water (*c.* 25 mg total hardness as $CaCO_3$ per kg), and analyses of tap-water from private homes and university laboratories gave, per kg cold water, 0·17–0·19 mg Fe, 0·02–0·07 mg Cu, 0·01–0·78 mg Zn; and per kg hot water, 0·19–0·60 mg Fe, 0·09–0·46 mg Cu, 0·12–0·78 mg Zn. Similar values for Cu were obtained by Macdonald (1963). Thus, if tap-water had been used for making the beverages, the daily intake might have been increased for subject D, who drank the largest volume of fluids, by up to 0·3 mg Fe, 0·1 mg Cu and 1·4 mg Zn if water from the cold tap had been used, or considerably more Fe (1 mg Fe) and Cu (0·8 mg Cu) if water from the hot tap had been used. Fe, Cu and Zn were all poorly absorbed, which suggests that a deficiency would be more likely to arise from failure in absorption or from increased losses than from an inadequate intake. The lipid content of the faeces in this study was very low (Swindells *et al.* 1968), so that there should have been little interference from lipids in the absorption of these cations.

Intake of Mo (48–96 μg/d) was close to that of 99–110 μg/d reported for three adults in longer balance studies of 30–70 d (Tipton, Stewart & Martin, 1966; Schroeder, Balassa & Tipton, 1970; Underwood, 1971), but was much less than those of 210 and 460 μg for two further subjects of Tipton, Stewart & Dickson (1969). The Mo content of foods varies considerably and, of the constituents in the meat loaf, legumes are classified as a rich source, whereas muscle meat, refined cereals and root vegetables are among the poorest. Liming the ground and fertilizing with superphosphate with or without added trace elements are commonly practised in New Zealand. The difference in Mo concentration of the meat loaf in the two studies was not accompanied by comparable changes in Mn or Cu concentrations. Two of the three subjects of Tipton *et al.* (1966) lost more Mo in the urine than in the faeces. A similar difference in excretion was seen in our subjects between the 1966 and 1967 study, but the reason is not apparent, particularly as the lowered intake was associated with a greater output in the faeces.

Little is known of the Se intake of people living in non-seleniferous areas. The intake of our subjects was 18–26 μg Se/d, about one-third of that listed by Schroeder & Nason (1971). The low intake was not surprising since New Zealand has many areas with low-Se soils, where the food of farm animals has had to be supplemented with Se. Moreover the first account of a possible Se deficiency occurring in man involved the use of New Zealand dried milk to treat Jamaican children suffering from kwashiorkor (Schwarz, 1961). The urinary output was also low, 7–16 μg Se/d, and was equivalent to about half the intake. Se is a difficult element to estimate accurately because of the many steps involved, susceptibility to contamination, and the presence of interferences. Urine is the least difficult biological material to measure and rough assessment of the Se intake may be obtained by doubling the value obtained for the Se content of a 24 h collection of urine (Thomson, 1972).

Even though the intakes and urinary outputs of trace elements in our study were often lower than those listed in Table 4 and Table 5 of the paper by Schroeder & Nason (1971), the urinary outputs of Fe, Cu, Zn and Se, expressed as a ratio of the

intake, are of the same order in both studies, whereas that of Cd was much higher in our study: 0·40 compared with 0·14. It is not clear how Schroeder & Nason (1971) derived their value of 0·14. Values of 0·42–0·83 were obtained for three subjects during long-term balance studies by Tipton *et al.* (1966, 1969).

Only one non-essential trace element was measured. This was Cd because of its alleged association with essential hypertension. Its intake (60–92 μg/d) was one-third of that listed by Schroeder & Nason (1971), who maintained that such a low intake could only be obtained from a vegetarian diet and pure water (Schroeder *et al.* 1967). The use of tap-water would have increased the intake by very little – less than 3 μg/l water. Our study showed that New Zealand diets tend to be low in Cd, high in protein and have a high Zn:Cd ratio (250:1) – all factors which are believed to depress absorption of Cd (Schroeder & Nason, 1971; Underwood, 1971). Lack of evidence of Cd retention in the balance study using both faecal markers suggest that there was little tendency for Cd to accumulate in these subjects. As regards extra dietary sources of Cd, New Zealand is a windy country with little industry to pollute the air and the subjects did not smoke cigarettes (Nandi, Jick, Slone, Jusko, Shapiro & Lewis, 1969; Lewis, Jusko, Coughlin & Hartz, 1972). The daily urinary output of Cd in this study is similar to that of ninety-six New Zealand students, 36 ± 23 μg Cd for forty-two men and 33 ± 21 μg Cd for fifty-four women (McKenzie, 1972*b*). Likewise, the daily urinary outputs of Zn were similar, $0{\cdot}58 \pm 0{\cdot}26$ mg Zn for the men and $0{\cdot}33 \pm 0{\cdot}17$ mg Zn for the women. The reason for the higher urinary excretion of subject W is not known, but her peak excretion was still within the range for normal people (Helwig, Hoffer, Thielen, Alcocer, Hotelling, Rogers & Lench, 1966; McKenzie, 1972*b*).

Balance studies are limited by the precision with which the intake and output can be determined (Hunscher, 1961; Walker, 1962; Hegsted, 1967; Isaksson & Sjögren, 1967; McCance, Rutishauser & Boozer, 1970). Balance studies of trace elements are particularly prone to errors because of the problems inherent in estimating small concentrations of trace elements in a variety of biological materials, with risks of contamination from the environment at all stages. Since many of the essential trace elements are poorly absorbed cations, it is especially important to relate faecal output to particular periods of feeding. The use of both continuous and intermittent markers reduced but did not eliminate apparent retentions, particularly of Fe, Cu, Mn and Zn. These retentions are believed to have resulted mainly from unmeasured dermal and menstrual losses. Balances for Mo, Se and Cd, which are more readily absorbed, showed smaller retentions if any. It is unlikely that any of the trace elements were in fact being retained, since the subjects had changed only the pattern of eating their meals, and this had already been shown not to alter their metabolic response (Swindells *et al.* 1968). The results have provided information about the intakes and individual variations of urinary and faecal outputs of six essential trace elements in four New Zealand women.

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