Intermittent and continuous faecal markers in short-term metabolic balance studies in young women

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1 The continuous faecal marker chromic oxide, Cr_2O_3, and the intermittent marker brilliant blue were used together in metabolic balance studies of calcium and magnesium in two young women.

2. Faecal output was derived by four methods: no marker, brilliant blue, Cr_2O_3, or both brilliant blue and Cr_2O_3.

3. Balances of Ca and Mg derived by the four methods of obtaining faecal output were compared.

4. Recovery of ingested Cr_2O_3 in the faeces appeared complete in experiments on three further subjects, with only a negligible amount being excreted in the urine.

5. Simultaneous use of both types of marker is recommended for short-term balance studies of nutrients such as Ca and Mg which are not readily absorbed.

In metabolic studies, it is necessary to identify and measure the faecal output from a particular intake of food. For nutrients such as sodium and potassium the faecal output represents such a small proportion of the intake that its measurement may even be neglected (Elkinton & Danowski, 1955), but the faecal output of nutrients which are not readily absorbed from the gastro-intestinal tract, such as calcium, magnesium and some of the trace elements, is considerable.

In most balance studies, particularly short-term studies, two types of faecal markers are used: an intermittent marker such as charcoal, carmine, or brilliant blue, which is administered at the beginning and end of an experimental period, thereby distinguishing the faeces related to that period of feeding (Lusk, 1931; McCance & Widdowson, 1942; Lutwak & Burton, 1964), and secondly a continuous marker such as polyethylene glycol, chromic oxide or barium sulphate, which defines the quantity of faeces related to a given quantity of food, assuming that the marker and the nutrient being studied have similar transit times through the alimentary tract (Whitby & Lang, 1960; Rampone, 1968; Figueroa, Jordan & Bassett, 1968; Borgström, Dahlqvist, Lundh & Sjövall, 1957). Davignon, Simmonds & Ahrens (1968) defined the criteria for an ideal marker, following their extensive investigation of the use of chromic oxide as an internal standard. Rose (1964) found the simultaneous use of carmine and chromic oxide particularly valuable in metabolic studies involving a change of diet or therapy (Hargreaves & Rose, 1965).

It is possible to measure the faecal output of a nutrient without using a marker, especially in long balance studies (Isaksson & Sjögren, 1967); the purpose of this investigation was to discover how much the measurement of faecal output could be improved by using the intermittent marker brilliant blue, the continuous marker

chromic oxide, or both together for the measurement of the metabolic balances of calcium and magnesium. Each marker revealed its own peculiar advantages in 27 d balance studies on two young women, and in order to derive the greatest benefit the markers needed to be used together. The criteria of Davignon *et al.* (1968) were applied to our studies, namely, that an ideal marker should be non-absorbable, non-toxic and easy to measure; it should be dispersed uniformly relative to the faecal constituent of interest in the particular investigation, and also a steady state should be attained rapidly in which, on the average, excretion and intake are equal. The recovery of ingested chromic oxide in the faeces was measured in three further subjects, as well as its excretion in the urine; the results indicated that a negligible amount of chromium had been absorbed.

EXPERIMENTAL

Balance study

The young women students, subject G and subject M, were two of the subjects in the balance study of metabolic effects of meal frequency, for which full details of diet and procedure have already been given (Swindells, Holmes & Robinson, 1968). The subjects ate a uniform diet for 27 d; the experimental regime 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). The same daily amount of food was divided into the appropriate number of equal-sized meals; 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 concentrations of Ca and Mg were measured in the diet and in the 24 h collections of urine.

Faeces

Two gelatin capsules, each containing 50 mg brilliant blue marker (FD & C Blue No. 1) and 200 mg methyl cellulose, were taken during the evening following the last meal of each period; the presence of green faeces marked the beginning of the next period (Lutwak & Burton, 1964). Chromic oxide (reagent grade Cr_2O_3; British Drug Houses Ltd) was purified to remove dichromate by the method of Whitby & Lang (1960). Gelatin capsules were filled by hand to contain 500 mg $\pm$ 2 mg Cr_2O_3 and one capsule was taken three times daily, with the three meals in periods 1, 3 and 5, and at about the same times of day in periods 2 and 4. Administration of Cr_2O_3 began on day 4 of period 1 and continued until day 3 of period 5; faeces were collected during this time until the brilliant blue taken at the end of period 4 had appeared.

Each stool was collected, weighed and dried under an infrared lamp, after which it was ground to a fine powder. The recorded weight of the stool included the weight of faeces on the toilet paper. Two-thirds of each stool was pooled for each period; the remainders of the stools were kept for analyses of separate stools.

Recovery of Cr_2O_3

Subjects R, H, and S swallowed three capsules of Cr_2O_3/d for 2 d, equivalent to a total intake of 3·0 g Cr_2O_3. Faeces were collected for those 2 d and the subsequent 10 d;

each stool was analysed for Cr and the recovery of ingested Cr_2O_3 determined. The factor used for converting Cr into Cr_2O_3 was 1·46.

Recovery of Cr from purified Cr_2O_3 was measured in six samples taken from capsules; the mean recovery was 101 % (range 100–103 %).

Excretion of Cr in urine

As in the previous experiment, subjects H and S swallowed three capsules of Cr_2O_3 daily for 2 d; 24 h urine samples were collected for 7 d, from the day before Cr_2O_3 was ingested until 4 d afterwards. Concentration of Cr was estimated in each day's urine.

For each period of the balance study, the concentration of Cr was measured in the pooled urines for subject G and subject M; the urines were pooled by mixing equal proportions of their 24 h volumes.

Analytical methods

Chromium was determined with a Techtron AA-100 atomic absorption spectrophotometer. Faeces were analysed by the method which Williams, David & Iismaa (1962) used for faecal samples in pasture experiments. Interference from small differences in the Ca and Mg concentrations of the samples was suppressed by spraying test and standard solutions containing 500 ppm Ca, but silicate was not added to standard solutions since human faeces contain little silicate (Sollmann, 1926). For urine, 200 ml were dried and ashed at 600°; Cr present was oxidized with HNO_3 and $KMnO_4$, extracted into isobutyl ketone and sprayed into the flame (Feldman, Knoblock & Purdy, 1967). Blank and standard solutions were similarly treated.

Ca and Mg were measured in the diet, faeces and urine by atomic absorption spectroscopy. Urines were analysed directly, but for the diet and faeces an acid extract of the ashed sample was first prepared. Interference by phosphate with the determination of Ca was suppressed by spraying solutions containing a 1 % concentration of lanthanum (Willis, 1961), and all solutions analysed for Mg were acidified to contain 0·1 N-HCl (Dawson & Heaton, 1961).

RESULTS

Faecal output

Brilliant blue was noticed first in the faeces of subject G on days 1, 3, 4 and 2 of periods 2, 3, 4 and 5, respectively, and in the faeces of subject M on days 2, 2, 5 and 8, respectively. These days marked the beginning of faecal collection during periods 2, 3, 4 and 5 for each subject. Table 1 gives the dried weight of faeces, the percentage dry matter and the concentrations of Ca, Mg and Cr_2O_3 in the pooled dried faeces for each period. Values for period 1 represent the composition of the faeces collected from day 4 until period 2 began, and those for period 5 represent the composition of faeces collected at the end of the study after the marker given at the end of period 4 had appeared. Fig. 1 gives the concentrations of Ca, Mg and Cr in the individual stools of the subjects; the concentrations of Ca and Mg in the dried faeces varied little throughout the experiment.

Table 1. *Weight and composition of faeces in young women for each period of the experiment*

(Period 1 was the preliminary period and period 5 the after-period)

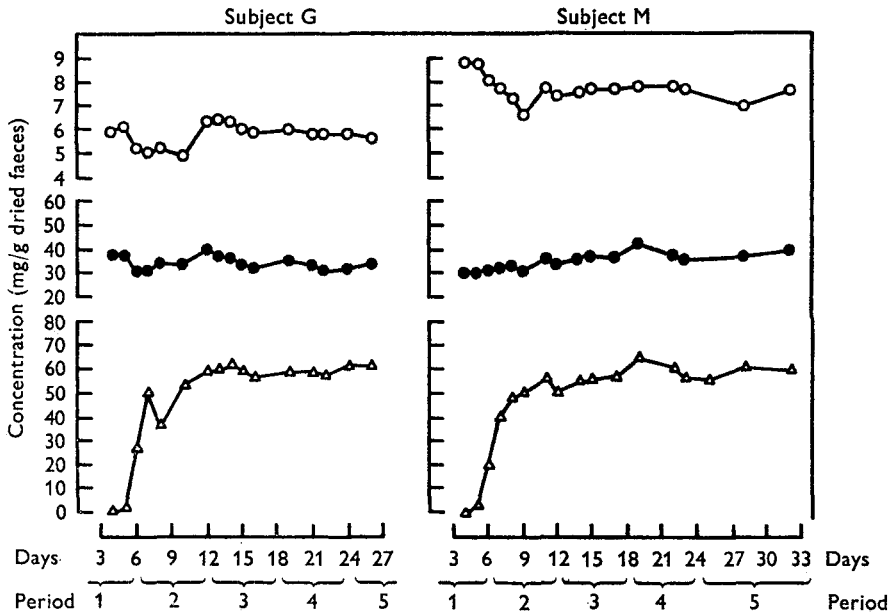| Subject	Period	Dried faecal weight (g/period)	Composition of faeces			
			Dry matter (% wet faeces)	Ca (mg/g dried faeces)	Mg (mg/g dried faeces)	Cr_2O_3 (mg/g dried faeces)
G	1	50	25	33	5·5	18
	2	114	27	32	5·6	85
	3	123	28	31	5·8	82
	4	61	29	32	5·7	90
	5	30	32	33	5·5	85
M	1	68	28	31	8·5	18
	2	80	30	35	7·5	78
	3	118	33	37	7·9	88
	4	35	32	36	7·8	92
	5	95	32	36	7·9	85

Fig. 1. Concentration (mg/g dried faeces) of magnesium (O), calcium (●) and chromium (△) in the individual stools of two young women.

In this study the faecal outputs of Ca and Mg have been estimated for the three experimental periods by four methods (Table 2).

(*a*) No marker: the faecal output was derived from the weight and composition of the faeces collected during the 6 d of each period.

(*b*) Brilliant blue: the beginning and end of faecal periods were 'marked', so that the faeces weighed and analysed were those produced from a particular period of feeding.

(*c*) Chromic oxide: the weight of faeces collected as in method (*a*) was adjusted by the ratio of the 9·0 g Cr_2O_3 ingested during each period to the amount of Cr_2O_3 recovered in the faeces.

(*d*) Brilliant blue and chromic oxide: the weight of faeces collected during each 'marked' period by method (*b*) was adjusted according to its content of Cr_2O_3 as in method (*c*).

The calculations were made for methods (*a*) and (*c*) from the results of analyses of individual stools, and for methods (*b*) and (*d*) from analyses of pooled stools. The dried faecal weight/d was obtained by dividing the dried weight/period by 6, the number of days in each period of feeding.

Table 2. *Daily faecal output of calcium and magnesium* (g) *by young women, derived by four methods of calculating dried faecal weight* (g)

Subject	Method of calculation	Dry weight in period			Ca in period			Mg in period		
		2	3	4	2	3	4	2	3	4
G	No marker	14	16	20	0·46	0·56	0·68	0·07	0·10	0·12
	Brilliant blue	19	20	10	0·61	0·64	0·33	0·11	0·12	0·06
	Cr_2O_3	18	17	17	0·66	0·59	0·57	0·10	0·10	0·10
	Cr_2O_3 + brilliant blue	18	18	17	0·56	0·57	0·53	0·10	0·11	0·10
M	No marker	15	10	15	0·53	0·36	0·57	0·11	0·07	0·12
	Brilliant blue	13	20	6	0·47	0·72	0·21	0·10	0·15	0·05
	Cr_2O_3	19	18	17	0·68	0·68	0·66	0·14	0·13	0·13
	Cr_2O_3 + brilliant blue	19	17	16	0·67	0·63	0·59	0·13	0·13	0·13

Table 3. *Daily intake and urinary excretion of calcium and magnesium* (g) *by young women in periods 2, 3 and 4*

(Mean values with standard deviations in parentheses)

Subject	Calcium				Magnesium			
	Intake	Urinary excretion in period			Intake	Urinary excretion in period		
		2	3	4		2	3	4
G	0·88	0·21 (0·02)	0·16 (0·02)	0·15 (0·05)	0·25	0·12 (0·01)	0·11 (0·02)	0·11 (0·03)
M	0·90	0·21 (0·02)	0·23 (0·03)	0·26 (0·03)	0·27	0·12 (0·02)	0·11 (0·01)	0·12 (0·01)

The daily dried weights showed considerable variation from period to period with method (*a*) and also with method (*b*), but this was reduced when the weights were corrected for the recovery of Cr_2O_3 in methods (*c*) and (*d*). Since the concentrations of Ca and Mg did not alter appreciably throughout the experiment (Fig. 1, Table 1), the variations in faecal weight were reflected in the variations in faecal output of Ca and of Mg (Table 2).

Table 4. *Magnesium balances (g/d) for young women, calculated by four methods of deriving faecal output*

Subject	Method of calculation	Mg balance in period		
		2	3	4
G	No marker	0·06	0·04	0·02
	Brilliant blue	0·02	0·02	0·08
	Cr_2O_3	0·04	0·04	0·04
	Cr_2O_3 + brilliant blue	0·03	0·03	0·04
M	No marker	0·04	0·09	0·03
	Brilliant blue	0·05	0·01	0·10
	Cr_2O_3	0·00	0·03	0·02
	Cr_2O_3 + brilliant blue	0·01	0·03	0·02
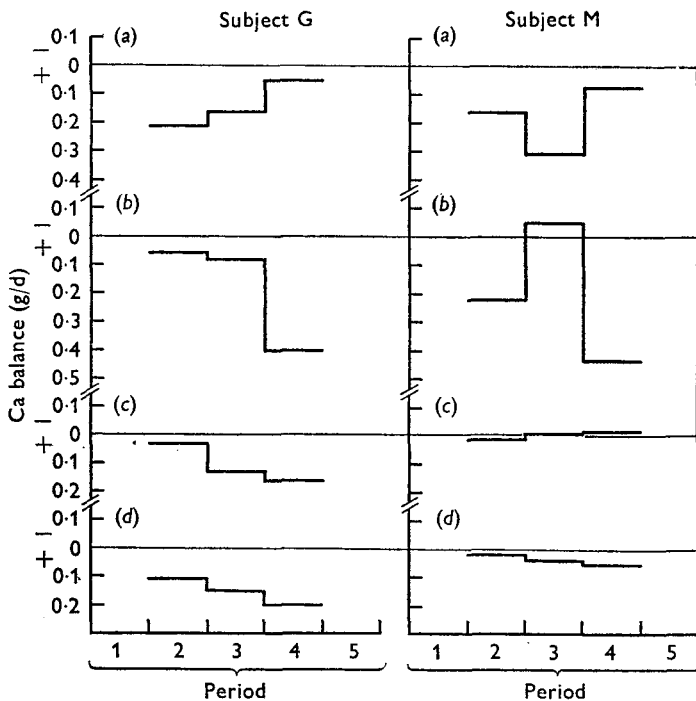
Fig. 2. Calcium balances for two young women calculated by the four methods of deriving faecal output: (*a*) no marker; (*b*) brilliant blue; (*c*) Cr_2O_3; (*d*) both brilliant blue and Cr_2O_3. Positive balances are plotted downwards.

Balance study

Table 3 contains the values for the intake of Ca and Mg by the two subjects. Subject M ingested more meat loaf and drank some of her daily allowance of fluid as black coffee, which accounted for her slightly greater intake of both Ca and Mg (Swindells *et al.* 1968). Mean urinary excretion of Ca and Mg for each period is also given in Table 3, and these values with those for the faecal output made up the 'total output' of Ca and Mg; no correction was made for dermal loss of either element.

The balance was estimated by the four methods of deriving the faecal output; the Mg balances are listed in Table 4, and the Ca balances are illustrated in Fig. 2.

Because the intake was constant and the urinary output varied little, the balances for Ca and Mg were largely influenced by the faecal weight for each period. In contrast to the balances obtained when no marker had been used, or when brilliant blue was used alone, the balances derived with the aid of Cr_2O_3 agreed from period to period; this was especially noticeable for subject M. Although the use of the intermittent marker as well as Cr_2O_3 did not alter the measured balance appreciably, it indicated the time taken for food to pass through the alimentary tract. This time increased as the study progressed, particularly in subject M for whom the last two doses of brilliant blue took 5 and 8 d to appear.
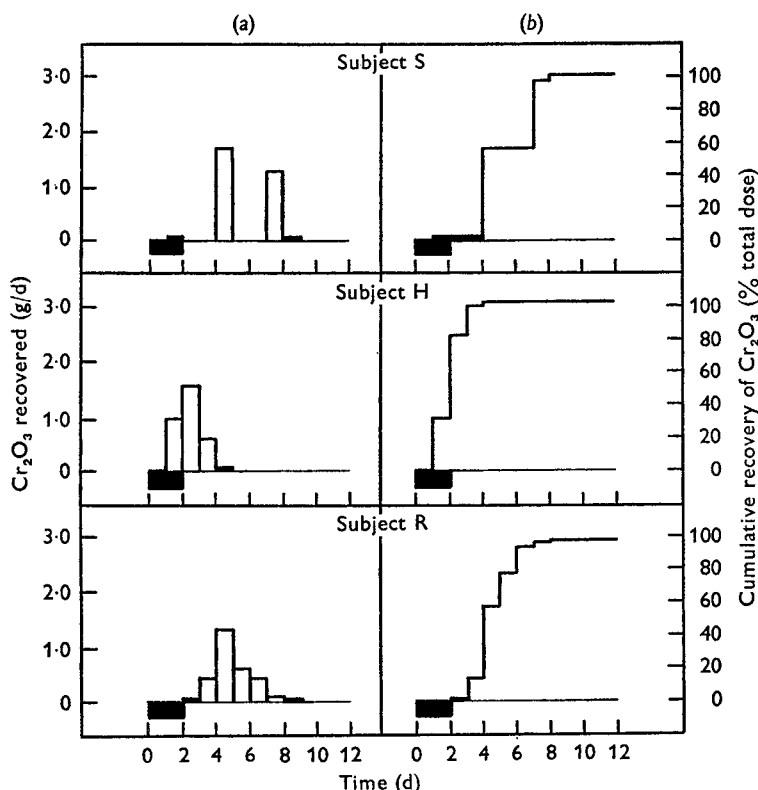

Fig. 3. Recovery of Cr_2O_3 in the stools of three young women during the 2 d (■) Cr_2O_3 (1·5 g/d) was ingested and the subsequent 10 d. (a) amount recovered; (b) cumulative recovery.

Recovery of Cr_2O_3

Although the use of Cr_2O_3 improved the reproducibility of the Ca and Mg balances in the three periods, the total recovery of Cr_2O_3 was incomplete, 83% for subject G and 85% for subject M. Whitby & Lang (1960) reported a mean recovery of 93% for six patients (range 88–98%), and later Rose (1964) obtained the same value (93%) for his twenty-one studies (range 80–103%). Both workers suggested that incomplete

collection of faeces was the cause, with possibly some loss on the toilet paper. In our study, faecal matter left on toilet paper was carefully measured and it was usually less than 0·1 g.

Fig. 3 illustrates the course of recovery of 3·0 g Cr_2O_3 ingested by subjects R, H and S. The pattern of elimination of faecal Cr_2O_3 differed in each subject; for subject R who had daily stools the transit time of Cr_2O_3 was long, for subject H it was short, and subject S tended to be constipated. No chromic oxide was detected in faeces of subject H 4 d after ingestion, but both subjects R and S had Cr_2O_3 in their faeces 3 d later on day 9. The recoveries were 97, 100 and 104% for subjects R, S and H, respectively. Considering that these values were obtained from the sum of many measurements and that the recovery of Cr from purified Cr_2O_3 was 101%, it seemed reasonable to conclude that recovery of Cr_2O_3 had been complete.

Table 5. *Urinary chromium excretion following ingestion of Cr_2O_3 by young women*

| | | | Urinary Cr excretion | |
| | | Cr_2O_3 ingested | Concentration | Output |
Subject	Day	(g/d)	(μg/l)	(μg/d)
H	1	—	9	23
	2	1·5	7	20
	3	1·5	4	13
	4	—	4	8
	5	—	5	14
	6	—	9	25
	7	—	7	16
S	1	—	10	13
	2	1·5	13	19
	3	1·5	54	73
	4	—	14	11
	5	—	5	6
	6	—	11	16
	7	—	8	11

Urinary excretion of chromium

The concentration of Cr in the urine was so low that precise measurement was impossible in the balance study. Sufficient urine for each estimation (200 ml) could only be obtained by pooling urines for each period; concentrations of Cr in urine varied between 4 and 25 μg/l for both subjects, corresponding to an output of 4–25μg/d (see Table 6, Swindells *et al.* 1968). Table 5 gives the urinary excretion of Cr following ingestion of 3·0 g Cr_2O_3. The concentration of Cr (4–9 μg/l) and the amount excreted each day (8–25 μg) were low for subject H, for whom the transit time for Cr_2O_3 had previously been short (Fig. 3). In subject S, however, for whom the transit time was long, the concentration of Cr increased suddenly on the 2nd day of administration of Cr_2O_3 to a value four to five times as high, but even this was only slightly above the normal range for Cr excretion of 20–40 μg/l reported by Schroeder (1968).

Steady state of faecal output

Davignon *et al.* (1968) defined 'ideality of Cr_2O_3 excretion' as the achievement of a steady state in which not only had the Cr_2O_3 concentration in the faeces reached

Faecal markers

a plateau but also the mean daily output of Cr_2O_3 was 90% or more of the intake. Although the Cr_2O_3 concentration might have reached a plateau in some subjects from whom the cumulative recovery was less than 90%, they contended that the true steady state had not been reached since an appreciable amount of marker had been retained. It would be invalid to use Cr_2O_3 as a reference marker for correction of faecal weights in these 'non-ideal' subjects. Davignon *et al.* (1968) calculated the faecal Cr_2O_3 concentration, the Cr_2O_3 balance and the cumulative recovery of Cr_2O_3 for each period. Similar calculations have been made for subjects G and M from the composition of the
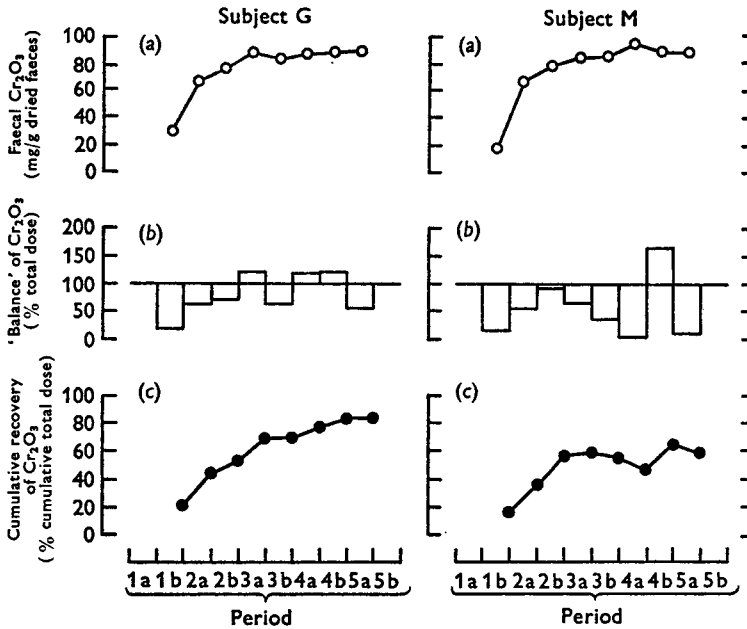

Fig. 4. Faecal excretion of Cr_2O_3 in two young women for each 3 d period. Cr_2O_3 was administered from beginning of period 1 b until day 3 of period 5 a.

individual stools but for 3 d periods, which is half the time of our 6 d experimental periods; these 'half-periods' have been designated as (*a*) and (*b*) corresponding to days 1–3 and days 4–6 of each period, respectively. Fig. 4 shows that for both subjects the Cr_2O_3 concentrations had reached a plateau by period 2 b. For subject G, the faecal excretion of Cr_2O_3 (or Cr_2O_3 balance) varied from 60 to 120% of the daily dose from periods 2 b–4 b and the mean daily output was 93% of the intake. However, for subject M, only in period 4 b was the excretion of Cr_2O_3 greater than the intake, and the mean daily output from periods 2 b–4 b was 84% of the ingested Cr_2O_3. The cumulative recovery increased steadily throughout the balance period for subject G and was more than 80% when ingestion of Cr_2O_3 ceased, whereas for subject M it remained almost unchanged from period 2 b onwards at 60% of the total dose.

If the criteria of Davignon *et al.* (1968) were applied in our study, subject G would be called an 'ideal' subject and subject M would not, with the inference that Cr_2O_3 was being retained. Referring back to Fig. 1, it will be seen that the excretion of

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Ca and Mg varied with that of Cr from day 4 and day 3 in period 2 for subjects G and M, respectively; correlation coefficients were calculated from these times until the ingestion of Cr_2O_3 was discontinued on day 3 of period 5 (Table 6). The significant correlations for subject M, and for subject G (with the exception of Ca and Cr), might suggest that Cr_2O_3 was uniformly dispersed relative to the faecal constituents (Davignon et al. 1968), making it permissible to correct for the incomplete collection of faeces in the balance studies of both our subjects. Furthermore, the low recovery of Cr_2O_3 had possibly been caused by a slowing down of bowel function as the study progressed; this was demonstrated by the use of brilliant blue.

Table 6. *Correlation coefficients for concentrations of calcium, magnesium and chromium in individual stools collected from young women during periods 2–5*

Subject	Correlation of	Correlation coefficient r	Significance P
G	Cr and Ca	0·39	NS (0·20)
	Cr and Mg	0·70	0·01
	Ca and Mg	0·68	0·02
M	Cr and Ca	0·96	< 0·001
	Cr and Mg	0·82	0·01
	Ca and Mg	0·75	0·02

NS, not significant.

DISCUSSION

Comparison of the results for the balances of Ca and Mg derived by the four methods confirmed the usefulness of Cr_2O_3 for measuring the faecal output. The question which now remains is whether it is an advantage to use an intermittent marker as well as Cr_2O_3.

First, by applying the criteria of Davignon et al. (1968) to the use of Cr_2O_3 alone in this study, it will be seen that Cr_2O_3 was easily estimated, and its recovery in the faeces appeared complete. The increase of 54 μg Cr in the urine of subject S, on the 2nd day that Cr_2O_3 was ingested, suggested that some Cr had been absorbed. This was not confirmed by the balance studies, but in these only 6 d pooled urines could be estimated. In his extensive review on chromium, Mertz (1969) stated that he did not know of any reports of oral toxicity of trivalent chromium. Moreover, Whitby & Lang (1960) as well as Davignon et al. (1968) reported no evidence of toxicity in their experience of using Cr_2O_3; the latter workers used a dose of 300 mg/d, one-fifth of that used by Whitby & Lang (1960) and in this study, and it is planned in future studies to use the smaller amount.

Scott & Humphreys (1962) were able to assess the dispersion of Cr_2O_3 along the alimentary tract of cats, after killing the animals. In our subjects the close association between the excretion of Ca, Mg and Cr in the faeces suggested that the Cr_2O_3 had become well mixed with the intestinal contents. Alvarez (1950) and Davenport (1961) supported the idea that each day about one-sixth of the material entering the colon passed through to be defaecated and was augmented by about half the contents of

the caecum. If such a pool of intestinal residues existed, Cr_2O_3 would have to be administered for many days before a state of equilibrium would be reached, since the Cr_2O_3 ingested in the first few days would be mixed with intestinal contents remaining from the previous 2 or 3 d. Likewise the elimination of the Cr_2O_3 after its administration ceased would be gradual, since the Cr_2O_3 in the intestinal pool would be diluted by incoming food residues. The existence of such a pool of intestinal residues seemed to be supported by several observations in our studies: quantitative recovery of the dose of Cr_2O_3 given on the 1st day took 3–4 d, although the marker was detected in the faeces on the 2nd day. Six days were required before a steady state was reached when the output of the marker equalled the intake, which confirmed the advisability of administering the marker for the entire length of the 6 d preliminary period, which unfortunately was not done in this study. Further, subject G, who was not noticeably constipated, retained between 4 and 7 g Cr_2O_3 from the 6th day of Cr_2O_3 administration, and the stools of subject R, who defaecated daily, still contained Cr_2O_3 7 d after the dose had been taken. Although there was no direct evidence that Cr_2O_3 had not been selectively retained it seemed more likely that the retention of Cr_2O_3, and also of Ca and Mg, was principally a simple consequence of constipation.

In short-term experiments there is uncertainty about whether a steady state has been attained and whether it is permissible to correct for variations in faecal output. It might seem preferable to analyse the exact faecal output from a particular period of feeding, but unfortunately this is impossible because of the mixing with residues from other periods. A rough method of demarcation can be achieved by the first appearance of an intermittent marker like brilliant blue, but this also becomes spread within the bowel (Lutwak & Burton, 1964). This method alone gave unsatisfactory results for Ca and Mg balances, although it gave some indication of the transit time of food through the alimentary tract. By using brilliant blue in addition to chromic oxide, the advantages of the two types of marker may be combined, and their combination probably permits the faecal output of Ca and Mg to be measured, as closely as is possible, in a metabolic balance study.

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