Urinary and faecal excretion of marker calcium (^{46}Ca) by low birthweight infants

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SUMMARY Low birthweight infants were given calcium enriched in ^{46}Ca in a single feed. The specific activity of successive urine samples showed that the absorption of the marker was largely complete in about 4 hours. The rate of decrease of urinary specific activity after $3\frac{1}{2}$ hours was exponential and very similar in 8 sets of observations in 6 infants. Its extrapolation backwards to 1 hour may indicate the size of the exchangeable calcium pool, usually about 200 mg/kg body weight. Completeness of faecal collection for estimation of ^{46}Ca is essential for accurate determination of true absorption and endogenous faecal excretion of natural Ca. In 5 infants examined, no marker was detectable in faeces excreted later than 48 hours after the first stool containing marker. Nevertheless, reasons are given why a collection period limited to 48 hours may sometimes involve error.

Endogenous faecal excretion E is the difference between true absorption from the intestine A and retention plus urinary excretion $R+U$. A marker for natural calcium added to a single feed enables A for calcium, and therefore E , to be measured; this was done in 9 low birthweight infants using ^{46}Ca as the marker (Barltrop *et al.*, 1977). With a feed of a given composition, A (intestinal absorption) was found to depend simply on calcium intake, but E (endogenous faecal excretion) was highly variable and seemed to be a characteristic of an individual infant. Investigation of the reasons for variability in E may throw light on important aspects of calcium metabolism in the infant. Successful use of the marker method depends on more than analytical accuracy, and this report deals with additional information obtained in the course of the pilot study referred to above. An important practical question is whether a collection period limited to 48 hours is adequate for urine and faeces.

Materials and methods

These were as described by Barltrop and Sutton (1972) and Barltrop *et al.* (1977). Three different milks were used differing only in their content of Ca and P. Calcium-46 marker was added to a single feed to which carmine was also added. 48 hours later carmine was added to a feed for the second time. The first faecal specimen to be collected was the

first to contain carmine and in the studies reported here all subsequent faecal specimens up to and including the second carmine-marked stool were retained and assayed individually. In these subjects all urine collections were also individually assayed.

A capillary blood specimen was taken by heel prick about 2 hours after the marked feed and collected into a heparinized tube. The greater part of the separated plasma was dried thermally, ashed, and used for measurement of ^{46}Ca . Natural Ca was measured in an aliquot of unashed plasma by atomic absorption spectrophotometry. The marker ^{46}Ca was measured by conversion into radioactive ^{47}Ca in a neutron flux and radioactive counting.

Results and discussion

Change in urinary specific activity with time after administration of marker. In the earlier studies each urine and faecal specimen was assayed separately. The Fig. shows how the urinary specific activity (SA), i.e. μg marker Ca per mg natural Ca, increased with time after the marked feed was given, reaching a maximum about 4 hours later and then falling exponentially for as long as observations were made. The time interval for a reduction of 50% in urine SA was in the range of 9-14 hours for 5 measurements on milk H and 10-11 hours for 3 measurements on milk M. (Milks H and M were identical except for their content of Ca: Barltrop *et al.*, 1977). The rate of fall of urinary SA with time showed that 48 hours was an adequate collection

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Fig. Variation of specific activity of plasma and urine with time after administration of marker. Time measured from midpoint of feed containing marker. Specific activity in μg marker per mg natural calcium. Case numbers are shown on the right. X, plasma values; O, urine values; plotted at midpoint of collection period ● Case 6 (2).

Calculated best fitting linear regression of log (urine specific activity) on time shown by broken lines extending over the range of points used for the calculation. In no case was there a significant difference between slopes calculated from $3\frac{1}{2}$ hours on and from 10 hours on. Where appropriate, broken lines are shown connecting up the points at earlier times. Cases 7b and 6 (1, 2) were on milk M, all the others on milk H.

period for urine and that errors resulting from failure to collect for longer would be small.

The urinary SA must be very close to, if not

identical with, the plasma specific activity during the time the urine was being formed in the kidney, and the SA of a urine sample is a reasonable representation of plasma SA at the midtime between it and the previous urine collection. This is illustrated in the Fig. which gives the directly measured plasma SA values as well as those for urine. Plasma SA was the same as urine SA at the same time except for Case 9 (1, 2). Since plasma Ca forms part of an exchangeable pool, an exponential decrease in plasma SA must represent a loss of marker calcium from that exchangeable pool into the tissues, principally bone, into the lumen of the gut, and into the urine. However, the infants were receiving 3 to 4 hourly feeds and those on milk H were absorbing 150 mg Ca/day (Table 1, col. 8, Barltrop *et al.*, 1977), a quantity several times greater than the amount of Ca in their extracellular fluid. (No information on A is available for the infants on milk M referred to in this paper.) Progressive dilution of marker calcium in plasma and extracellular fluid by 4-hourly feeding may be an additional factor underlying the progressive decrease of plasma SA with a half-time of 9–14 hours. The earliest urine samples showed that absorption was occurring by $\frac{1}{2}$ to 1 hour after the feed (Fig.) but the timing of the peak SA suggests that absorption continued at least until the next feed was given.

Renal clearance. Renal clearance, expressed as:

$$\frac{\text{Urinary Ca or } ^{45}\text{Ca excreted per unit time}}{\text{Ca or } ^{45}\text{Ca/l plasma}}$$

was estimated by relating the blood sample either to the urine specimen excreted immediately after the blood sample was obtained or to the sum of two urine specimens, that preceding and that succeeding the blood sample.

Renal clearances for natural Ca and for ^{45}Ca were closely correlated (Table 1). Reasonably similar values were obtained in repeat measurements on Cases 9 and 11. The fivefold variation in renal clearance between different infants has no immediate explanation. Renal and endogenous faecal excretions might be closely correlated since both are determined by the specific activity of the plasma, but this is not borne out by the data (Table 1). Variations between infants in active secretion of calcium into the intestine would diminish the correlation of E with renal filtration rates.

Exchangeable pool of calcium. As discussed by Aubert *et al.* (1963) and Marshall (1969), the exchangeable pool of calcium is not a well-defined physiological entity. Nevertheless, its magnitude as determined by calculating the dilution of a known

Table 1 Renal clearances of natural calcium and of ^{46}Ca marker

Case no.	Ca/100 ml plasma (mg)	Renal clearance (ml/24 h)		Endogenous faecal excretion† (mg/24 h)
		Natural Ca	^{46}Ca marker	
Milk M				
7a	8.6	22	26	—
7b	9.2	40	35	—
Milk H				
8	8.1	16	21	23
9 (1)*	9.7	69	50	46
10	10.7	11	10	126
9 (2)*	10.0	74	43	150
11 (a)	9.4	16	21	} 4
11 (b)	9.7	28	23	
Overall mean	9.4	35	29	

Note: All renal clearances were determined a few hours after administration of marker (cf. Fig.) except for Case 11 (b) which was at 24 hours after.

*Case 9 studied on 2 separate occasions.

†From Table 1, Barltrop *et al.* (1977).

amount of marker introduced into the blood may sometimes be of interest. Usually the marker is introduced directly by intravenous injection and measurements of plasma SA at successive intervals of time are found to fall on an exponentially decreasing curve which can be extrapolated back to zero time. The volume in which the marker is diluted is given by the ratio of SA of injected material to the derived SA of plasma at zero time. When a marker is given by mouth, absorption into the blood will be a slow process compared with an injection taking a few seconds. The plasma SA, instead of starting high and continuously decreasing with the lapse of time, is initially zero and then rises, though relatively slowly, to a peak value at a time when absorption is nearly completed and will then fall. After the peak is reached the plasma SA again decreases exponentially with the lapse of time and again the curve can be extrapolated backwards to the appropriate moment on the time scale corresponding to zero time for intravenous injection. In principle the magnitude of the exchangeable pool of calcium could be deduced as after intravenous injection, given a knowledge of how much of the orally administered material had been absorbed.

The main difficulty in applying this method to the data obtained in infants given a single feed marked with ^{46}Ca lies in the choice of the appropriate time to which to extrapolate plasma SA. Other possible sources of error seem to be unimportant: absorption from the gastrointestinal tract appeared to be complete (Barltrop *et al.*, 1977) and urine SA can be regarded as a good measure of plasma SA, as already noted. After oral administration, absorption into the blood is not likely to begin until after some delay and it is not likely to have the kinetics of simple nonexchangeable transfer from one compartment (the lumen of the upper intestinal tract) to

another (blood and extracellular fluid). As a compromise the time chosen for extrapolation was 1 hour after the midpoint of administration of the marked feed which in each case was completed within 30 minutes. Values of exchangeable Ca determined in this way are given in Table 2. Values derived from the SA at $\frac{1}{2}$ or 2 hours are similar. A practical problem is to know which urine SA values to exclude from the calculation of the best fitting exponential slope. After inspection of the data (Fig.) all values obtained before $3\frac{1}{2}$ hours were excluded and all points after and including those at $3\frac{1}{2}$ hours were used.

The data for 8 observations on 6 infants are given in Table 2. 5 infants gave values for the exchangeable pool of Ca in the range 140–200 mg/kg body weight. The sixth infant (Case 9) gave values twice as great on two occasions. The unusual features of both studies on this infant as compared with the other subjects (Fig.) were the higher value of plasma SA than of urine SA at the same time and the fact that the extrapolated line relating log urine SA and time went so close to the measured value of plasma SA. The data of Table 3 may suggest that the marker was exceptionally rapidly excreted into the bowel on each of the two occasions. However, there is really no explanation of how plasma SA could be so much greater than urine SA, and the values of exchangeable Ca for Case 9 (1) and (2), derived from urine SA (Table 2) must be suspect. Excluding the values for Case 9 (1) and (2) a mean value of 190 mg exchangeable Ca/kg body weight is obtained for these low birthweight infants.

A somewhat greater value of 312 mg Ca/kg body weight was given by Hoffenberg *et al.* (1964) for normal infants of mean weight about 9 kg and likely to be more mature than the infants studied here with body weights about 2 kg.

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Table 2 Exchangeable calcium pool*

Case no.	Weight (kg)	Marker absorbed (μg)†	SA urine at 1 h μg marker/mg Ca	Exchangeable Ca pool	
				mg	mg/kg
Milk M					
7b	2.7	1410	3.7	380	140
6 (1)	1.9	664	1.7	390	200
6 (2)	2.0	724	1.8	400	200
Mean				390	180
Milk H					
8	2.4	600	1.2	500	210
9 (1)	1.9	740	0.86	860	450
10	2.3	680	1.9	360	160
9 (2)	2.1	660	0.87	760	360
11	1.5	680	2.2	310	210
Mean				560	280

*Exchangeable pool, $\text{mg} = \frac{\text{amount of marker given } \mu\text{g} \times \text{fraction absorbed}}{\text{SA plasma } \mu\text{g marker/mg Ca}}$. Intake of marker 2000 μg , true absorption from Table 1 (Barltrop *et al.*, 1977), SA plasma taken to equal SA urine at 1 hour determined by extrapolation from calculated regression of SA urine on time since administration of marker (Fig.).

†For subjects on milk M values for true absorption A are not available and net absorption ($R + U$) is taken as the nearest approximation. Thus the values of the exchangeable pool for these subjects will be erroneously low in proportion to the magnitude of their endogenous faecal excretion since $A = R + U + E$.

Table 3 Faecal and urinary content of marker for collection times other than 48 hours and the corresponding values of A and E for natural calcium

Case no.	Time after intake T_1 hours	Marker			Natural Ca collected over T_0-T_1 hours	
		Collected over T_0-T_1 hours		Endogenous faecal excretion (calculated %) $\dagger$		
		Faeces (mg)	Urine (μg)		A (mg/d)	E (mg/d)
Milk M						
5	48	1.45				
	Later*	No additional marker detected‡				
7a	31	0.57				
	48	0.76				
7b	12	0.52				
	48	0.61				
Milk H						
8	28	1.18	0.8	2.5	209	90
	48	1.43	1.2	1.1	142	23
9 (1)	14	1.12	4.0	3.2	205	90
	48	1.30	4.6	1.8	161	46
	Later*	No additional marker detected‡				
10	32	0.17	1.6	21.8	582	544
	48	1.44	2.0	6.3	171	126
	Later*	No additional marker detected‡				
9 (2)	21	1.38	2.4	3.0	166	157
	48	1.42	3.0	3.6	159	150
	Later*	No additional marker detected‡				
11	33	1.33	3.7	0.3	111	3.7
	48	1.33	4.0	0.3	111	4.0
	Later*	No additional marker detected‡				

*2nd carmine-labelled stool.

†The same values for daily intake and excretion of natural Ca were used for each calculation on a given subject.

‡ $<10 \mu\text{g}$ (see text).

Completeness of faecal collection of marker. The deductions about A and E are made using the equations of Aubert *et al.* (1963). These state that

$$\text{Specific Activity of Urine} = \text{Specific Activity of Intake} - \frac{1}{E} \left[\text{Specific Activity of Intake} \times \text{Faecal content of natural Calcium} - \text{Faecal content of marker} \right] \quad (1)$$

where E = endogenous excretion into the bowel. The intake is a single feed containing marker and its specific activity is therefore unambiguous. Specific activity of urine and faeces, however, must depend on the duration of collection of urine and of faeces, since output of marker steadily decreases with time. The output rate of natural calcium is assumed to be constant both in urine and faeces. As equation (1) shows, any underestimate of the faecal content of marker will lead to an overestimate of E , and, because $\text{retention} = A - (E + U)$, to an overestimate also of A , the true absorption of natural calcium. Small errors in faecal content of marker lead to proportionately larger errors in A and E , as the equation above shows, because the specific activity of urine will always be only a few per cent of the specific activity of the intake in the marked feed.

There are no difficulties in principle in collecting all the urine excreted in the 48 hours succeeding the marked feed while avoiding contamination by urine earlier or later than this but faecal collection is less straightforward. In order to ensure as far as possible the completeness of faecal collection, a carmine marker was given in the feed containing the marker and again in the feed given exactly 48 hours later. The faecal samples from the first marked with carmine to the last before the second sample marked with carmine taken together therefore represent the physiological equivalent of 48 hours excretion whatever the actual interval of time over which they were collected or the rate of intestinal transit.* Nevertheless, the second specimen containing carmine might contain some faeces belonging to the intended collection period depending on how the intestinal contents are mixed in their passage through the gut. This is a source of little error where natural calcium estimations are concerned since a similar mixing can be expected to affect the first carmine-marked specimen. On the other hand it could be a specific source of underestimation of faecal output of calcium marker.

Table 3 shows how the cumulative total of faecal marker increased with time after the marked feed had been given. In 4 of the 8 cases as much as 20% or more of the marker was excreted in the last of the specimens making up the 48-hour collection. Nevertheless, no marker was detected in later specimens in the 5 cases examined at later times. The proportionately large effect on A and E of small deficiencies in faecal marker is shown in Table 3 for Cases 9 (1) and 8 where the last specimen examined contained 14 and 17% respectively of the

total marker excreted. Omitting these led to an increase in E by a factor of 2 and 4 and in A by a factor of 1.3 and 1.5 respectively. The calculated values for endogenous faecal excretion of the marker were also changed by large factors. The possibility of errors arising from incomplete faecal collection is emphasized by Case 10 who excreted 90% of his marker in the last of the specimens making up his 48-hour collection. His data would certainly have been suspect, especially because the endogenous faecal excretion of the marker was relatively large, had not his faecal specimens over a further 24 hours been separately analysed.

It may seem surprising that marker was no longer detectable in the faeces after 48 hours, where it had been present in mg amounts, whereas it could be detected in urine in fractions of $1\mu\text{g}$. The reason is that ^{46}Ca is present to the extent of $\sim 3\mu\text{g}$ in every faecal specimen quite apart from marker administration in the feed because its natural abundance is about 3×10^{-5} , that is about $3\mu\text{g}$ ^{46}Ca in every 100 mg of natural Ca. The marker contained 3.5% ^{46}Ca (Barltrop and Sutton, 1972) so that when 2 mg was given in a marked feed the amount of ^{46}Ca given was $70\mu\text{g}$. Urine assays are two orders of magnitude more sensitive than faecal assays simply because the mass of natural calcium in a urine collection is two orders of magnitude less than in a faecal sample.

In retrospect it would have been an improvement in the method always to have analysed the second carmine stool in order to show that no marker was detectable and therefore that there was no methodological defect. The results in Case 3b where this was not done (and which are not included in Table 3 because all his faecal specimens were pooled) may be regarded as probably in error because of delayed faecal excretion of the marker (Barltrop *et al.*, 1977). In this subject the calculated endogenous faecal excretion of marker was very high (9.3%) and A and E for natural calcium were also each anomalously high.

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*The diagram of the procedure in Barltrop and Sutton (1972) applied only to the first 2 infants examined when the overall collection period for natural calcium was 72 hours and for marker calcium was 48 hours.

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