Brit. J. Nutr. (1965), 19, 111

Strontium balance in breast-fed babies

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(*Received* 9 *September* 1964—*Accepted* 9 *October* 1964)

We have already reported that, in 3-day balance studies on male breast-fed babies between the 6th and 9th day after birth, more strontium was excreted than ingested, though the balance for calcium and phosphorus was positive (Widdowson, Slater, Harrison & Sutton, 1960). More recently, measurements of the Ca, Sr and phosphate balances in older bottle-fed infants have been reported by others (Lough, Rivera & Comar, 1963; Straub, Kahn, Wellman, Telles & Seltzer, 1964). In these investigations, the infants were from 4 to 7 weeks old. For Sr, the results were difficult to interpret because in the earlier study tap water, which contains Sr, was accidentally used as a dietary additive before the experimental period, and in the later study the diapers used for the collection of the urine and faeces had a relatively high Sr content.

Interest in mineral balance in the newborn, particularly that of Sr (Comar, 1961; Loutit, 1960), has stimulated us to extend our original observations, which are, so far as we are aware, unique not only because the infants were breast-fed, but also because separate values were obtained for the intake as well as the urinary and faecal output of Sr in the very young.

The Sr and Ca balance for three babies 6 weeks old was also measured, so that the net retention and absorption of Ca could be compared in the two age groups.

EXPERIMENTAL

Subjects

The babies were born at the Maternity Hospital, Mill Road, Cambridge. They had been fully breast-fed from birth. Each infant was weighed before and after each feed during the 3-day balance trials, and the milk intakes were obtained by difference. There was no regurgitation.

Breast milk was expressed from each mother during the balance study for the determination of Sr and Ca. The method used for the collection of urine and faeces passed by the babies has already been described (Slater, 1961).

Analytical methods

General. In our earlier experiments the breast milk was analysed for Sr when sufficient could be obtained from the mother. Thus, analyses were made for four out of nine nursing mothers during the balance study. Sufficient milk was obtained from all the mothers for the determination of Ca. The ratio, Sr:Ca was also determined in a pooled sample from fifteen other mothers. When the Sr intake was not determined directly, it was calculated from the total Ca intake of the infant and the mean value of the ratio Sr:Ca.

In the measurements reported here, about 30 ml of milk from each mother, about one-third of the bulked faecal output and portions of about 300 ml from the bulked urine passed over 3 days by each infant were reserved for Ca and Sr determinations. The milk and faecal homogenates were dried and thermally ashed, and the ash was dissolved in dilute hydrochloric acid.

Ca was determined, as before, by a modification of the method of Clark & Collip described by Economou-Mavrou & McCance (1958).

Sr determinations. Sr with Ca was precipitated from milk, faecal ash or a concentrate of acidified urine by adding excess of ammonium oxalate, the pH being adjusted to about 7 by adding ammonium hydroxide. The resulting precipitate of mixed oxalates was chilled for 2 h, separated, and washed with dilute ammonium hydroxide before being dried.

Sr in the oxalate extracts was determined by one of two different methods, depending on the amount of Sr present. In the samples of faecal ash, which contained more than 5 μg of Sr, the determination was carried out by the additive standard flame-spectrophotometric method described by Harrison (1958). The faecal oxalates were redissolved in nitric acid and Sr was reprecipitated as strontium nitrate further to purify the extract before the measurement. For milk and urine samples, which contained only 1 or 2 μg of Sr, the measurement was carried out by neutron activation analysis. The procedure was similar to that described by Harrison & Raymond (1955). A weighed portion of oxalate extract from milk or urine was irradiated for about $2\frac{1}{2}$ h in a neutron flux of about 10^{12} per cm^2 per sec at the same time as a weighed amount of pure strontium carbonate. In all analyses the chemical yield of Sr was determined radiochemically by adding ^{85}Sr (about 150 pc) to the original sample. The ratio of the final to the initial count-rate of ^{85}Sr was used to assess the chemical recovery.

'Blank' determinations were made for Sr on washings from the equipment used to collect the excreta and on the carmine marker used in the faecal collection. The Sr content of each of these was less than 1% of that in the relevant sample.

RESULTS

Table 1 gives the milk intake and urine output in ml for each of twelve babies over the 3-day balance study from the 6th day after birth and for three babies 6 weeks old. The concentrations of Ca and Sr in the milk and urine of each baby are also shown in the table.

There was considerable range in the observed values, especially for the Ca and Sr concentrations in the urine. However, the ratio of Sr to Ca in milk and urine showed a smaller range, mean values and their standard errors being 0.24 ± 0.02 (milk) and 1.16 ± 0.16 (urine) for the younger group of babies.

The Ca and Sr concentrations of mother's milk did not appear to change between 1 week and 6 weeks after birth, and changes in the Ca and Sr concentration of infant urine with age were equivocal.

Table 1. *Total milk intakes and urinary outputs of breast-fed babies, and calcium and strontium concentrations in milk and urine*

Infant	Milk: Total intake (ml)	Milk: Ca (mg/100 ml)	Milk: Sr (µg/100 ml)	Milk: Ratio, (Sr:Ca) × 10³
Age 6–8 days				
A	1533	26·0	6·6	0·25
B	1294	37·4	6·8	0·18
C	2115	22·8	3·9	0·17
D	1096	37·3	7·8	0·21
E	1932	34·5	4·7	0·14
F	1742	32·4	9·3	0·29
G	1720	30·7	8·6	0·28
H	1380	25·0	8·4	0·34
I	916	32·3	8·9	0·28
J	1415	22·0	4·7	0·21
K	1403	28·2	8·8	0·31
L	1320	34·4	9·1	0·26
Mean	1489	30·3	7·4	0·24
Range	916–2115	22–37·4	3·9–9·3	±0·02
Age 6 weeks				
B	1999	31·8	9·6	0·30
C	2606	27·9	7·8	0·28
M	2052	26·2	5·6	0·21
Mean	2219	28·6	7·7	0·26
Range	1999–2606	26·2–31·8	5·6–9·6	—

Infant	Urine: Total output (ml)	Urine: Ca (mg/100 ml)	Urine: Sr (µg/100 ml)	Urine: Ratio, (Sr:Ca) × 10³
Age 6–8 days				
A	1153	2·9	3·1	1·07
B	722	1·7	1·7	1·00
C	1071	3·8	2·8	0·74
D	649	0·73	1·1	1·51
E	1047	1·5	1·2	0·80
F	954	2·2	5·4	2·46
G	1036	5·6	10·3	1·84
H	760	2·4	2·6	1·08
I	515	6·5	4·4	0·68
J	720	2·3	2·8	1·22
K	940	4·3	3·0	0·70
L	782	4·1	3·4	0·83
Mean	862	3·2	3·5	1·16
Range	515–1153	0·7–6·5	1 1–10·3	±0·16
Age 6 weeks				
B	848	1·0	1·2	1·2
C	1405	7·5	3·5	0·47
M	1029	5·1	3·2	0·63
Mean	1094	4·5	2·6	0·58
Range	848–1405	1·0–7·5	1·2–3·5	—

Table 2. *Calcium and strontium 3-day balances for breast-fed babies*

Infant	Body-weight (kg)	Intake		Output: Urine		Output: Faeces		Retention		Absorption	
		Ca (mg)	Sr (μg)	Ca (mg)	Sr (μg)	Ca (mg)	Sr (μg)	Ca (mg)	Sr (μg)	Ca (mg)	Sr (μg)
Age 6–8 days											
A	3·40	398	101	33·6	35·9	234	70	130	−5	164	31
B	4·01	485	88	12·2	12·2	268	70	205	6	217	18
C	3·55	484	83	40·5	29·8	136	37	307	16	348	46
D	3·15	410	85	4·7	7·2	188	58	217	20	222	27
E	4·34	667	91	15·6	12·5	372	101	279	−22	295	−10
F	3·79	565	161	20·5	51·5	314	115	230	−5	251	46
G	3·25	527	147	57·5	107	195	88	274	−48	332	59
H	2·89	345	116	18·2	20·0	230	107	97	−11	115	9
I	3·21	296	82	33·7	22·6	85	43	177	16	211	39
J	3·00	311	67	16·2	20·0	162	62	133	−15	149	5
K	2·78	397	124	40·3	28·2	222	170	135	−74	175	−46
L	3·15	455	120	32·0	26·6	322	167	101	−74	133	−47
Mean	3·38	455	105	27·1	31·1	227	91	190	−16	218	15
Range	2·78–4·34	296–667	67–124	4·7–57·5	7·2–107	85–322	37–170	97–307	−74 to 20	115–348	−47 to 59
Age 6 weeks											
B	4·75	635	192	8·5	10·4	292	72	335	110	343	120
C	5·10	727	204	105	48·5	272	62	350	94	455	142
M	3·44	537	115	52·6	33·3	231	55	253	27	306	60
Mean	4·43	633	170	55·4	30·7	265	63	313	77	368	120
Range	3·44–5·10	537–727	115–204	8·5–105	30·4–48·5	231–292	55–72	253–350	27–110	306–455	60–120

Table 2 shows the body-weight in kg, with the intake and the urinary and faecal outputs of Ca and Sr, over the 3-day balance period for each baby. The retention and apparent absorption of Ca and Sr are also shown. Retention is expressed as intake minus total output (urine plus faeces) and apparent absorption as intake minus the faecal output. Both retention and the apparent absorption were thus derived as differences between two relatively large quantities. In each of the latter there was a considerable range for the different babies, so that it is not surprising that there was a fairly wide distribution in the values obtained for the retentions and apparent absorptions of Ca and Sr. However, for the younger group, in only four out of twelve was the retention of Sr positive. The mean retention was $-16\mu g$ over the 3-day period, or about $-1\cdot7\mu g/kg$ day. For each of the babies 6 weeks old, however, there was a positive retention of Sr, the mean being $+77\mu g$, or $5\cdot8\mu g/kg$ day. The retention of Ca was markedly positive in all of them.

DISCUSSION

The mean urinary outputs and body retentions of Ca by breast-fed babies as reported by us earlier (Widdowson *et al.* 1960) were 5·3 and +20 mg/kg day and 14 and $-20\mu g/kg$ day for Sr, compared with 2·7 and +19 mg/kg day for Ca and 3·1 and $-1\cdot7\mu g/kg$ day for Sr in the measurements reported here.

In this study, the urinary output of Sr for breast-fed babies was determined by neutron activation analysis. It is our experience that this more sensitive but more laborious method of assay is necessary when the sample contains less than $5\mu g$ of Sr (Harrison, 1958). However, in the earlier assays the Sr content of the urine samples from the breast-fed babies often appeared to contain more than $5\mu g$ Sr, so that the assays were made by flame spectrophotometry. A large 'off-line' reading was observed on the spectrophotometer in the measurement of the Sr emission from these samples. We regard the present measurements of the Sr content in urine by neutron activation analysis as more accurate than the values found earlier, and the smaller negative Sr balance now obtained for the breast-fed babies is thus regarded as more reliable.

One possible artifact that would produce a negative balance would be the presence of meconium, found to contain 2·7 ppm of Sr (Widdowson, McCance, Harrison & Sutton, 1962), in the faeces. But to account for the mean negative balance for Sr ($-16\mu g$ in 3 days) the meconium content of each day's faeces would have to be 2 g. Although meconium was visible in certain stools even at 6 days after birth, it is improbable that the daily output approached this level.

In Britain, over the last few years, bones taken at autopsy from babies of various ages have been assayed for stable Sr and Ca as part of the national survey of any internal contamination of the population by ^{90}Sr. The results have been reviewed recently by Bryant & Loutit (1964), who conclude from the data for infants 0- to 1-day-old dying during 1959–61 that the ratio for Sr:Ca in bone is significantly greater in the London area than in Wales or Lancashire. Mole (1965) has emphasized further the dependence on the place of birth of the Sr:Ca ratio in bone of the newborn and the change in the ratio with age. A recent analysis of the results for the ratio

Sr:Ca in the bone of infants born in the London area between 1957–60 and 1960–2 by R. H. Mole (1964, personal communication) is shown in Table 3. These results suggest that there is a slight fall in the ratio during the first few days after birth, followed by a rise in the ratio for infants more than 8 days old. There is no record of how many of these babies were breast-fed. It was shown in the original investigation (Widdowson *et al.* 1960) that bottle-fed babies were in positive balance for Sr as well as Ca, so that the ratio of Sr to Ca would be expected to increase for such infants. Thus the slight fall in the ratio shown in Table 3 during the 1st week after birth might well have been greater had all the babies been breast-fed.

Table 3. *Mean values with their standard errors for the ratio of strontium to calcium (expressed as μg/g) in bone from babies born in the London area*

Year of birth	Stillborn	0–2 days old	3–7 days old	8–30 days old
1957–60	221 ± 12 (24)	206 ± 17 (10)	215 (2)	197 ± 7 (3)
1960–2	261 ± 17 (16)	219 ± 12 (19)	288 ± 43 (4)	308 ± 23 (6)

The numbers of subjects are given in parentheses.

Table 4. *Calcium and strontium concentrations and the ratios of these concentrations in tissues, gut and contents of stillborn babies*

Stillbirth	Body-weight (kg)	Tissue	Ca (ppm wet weight)	$Sr \times 10^3$ (ppm wet weight)	Ratio, $(Sr:Ca) \times 10^3$
1 (anencephalus)	0·88	Muscle	89	49	0·55
		Lung and spleen	82	40	0·49
		Bone	134×10^3	20×10^3	0·15
		Gut and contents	292	96	0·33
2	2·95	Bone	106×10^3	21×10^3	0·20
		Gut and contents	206	85	0·41
3	3·46	Bone	119×10^3	22×10^3	0·19
		Gut and contents	204	270	1·3
4	2·64	Stomach, etc.	171	80	0·47
		Muscle	93	35	0·38
		Placenta	182	66	0·36
		Bone	124×10^3	22×10^3	0·18
		Lower gut and contents	455	220	0·48

We have recently measured the Sr, Ca and P contents of various tissues taken from stillborn babies. The Ca and Sr concentrations, as well as the ratio of Sr:Ca in bone, gut and contents and soft tissue, are shown in Table 4. It will be seen that the ratio Sr:Ca in bone varies from $0·15$ to $0·20 \times 10^{-3}$, but that the ratio in other organs ranged from $0·34$ to $0·55 \times 10^{-3}$ and that for the gut and contents from $0·41$ to $1·3 \times 10^{-3}$. It is therefore possible that the relatively high Sr:Ca ratios in soft tissues are due to relatively high concentrations of Sr in foetal blood compared with that in foetal bone. High concentrations of Sr in foetal and maternal blood have also been reported by Rivera (1963). Any 'excess' Sr in foetal blood would be excreted in urine during the early postnatal period. By reference to Table 1 it will be seen that the mean ratio of Sr:Ca in urine of babies 6–8 days old was $1·16 \times 10^{-3} \pm 0·16$, whereas for the 6-week-old babies it was $0·58 \times 10^{-3}$. If renal discrimination against Sr is unchanged in the first 6 weeks after birth, these results must mean that excess Sr is being eliminated in the urine of the 6–8 days old babies. Although a change in the output of Sr in the urine was detectable in breast-fed babies, it would not be measurable in bottle-fed infants for whom the daily intake of both Sr and Ca was several times greater.

SUMMARY

1. The calcium and strontium intakes and the urinary and faecal outputs have been measured over 3 days in twelve babies from the 6th day after birth and in three babies who were 6 weeks old.

2. The mean Sr:Ca ratio in the human milk received by the younger group of babies was $0.24 \pm 0.02 \times 10^{-3}$ and in the urine of the newborn $1.16 \pm 0.16 \times 10^{-3}$. For the babies 6 weeks old, this milk ratio was 0.26×10^{-3} and that for urine 0.58×10^{-3}.

3. The mean negative balance for Sr was $-1.7\ \mu g/kg$ day for babies between 6 and 8 days old, although the Ca balance was always positive. The 6-week-old babies were in positive balance for Sr as well as for Ca.

4. The negative balance for Sr was smaller than that obtained in an earlier study. Improvements in the assay of Sr, particularly in urine, are described and make the new results more reliable.

5. The negative Sr balance observed in young babies is not inconsistent with the results obtained for the Sr:Ca ratios in bones from stillborn babies or those dying in the first few days after birth.

6. A possible reason for a high urinary output of Sr in the newborn is discussed.

We would like to thank Dr Mole for providing the figures given in Table 3 and Professor R. A. McCance, FRS for his assistance with the preparation of the script. Our special thanks are also given to Miss S. H. McKeag, who was responsible for all the measurements and collections from the babies at the Maternity Hospital.

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Printed in Great Britain