INTAKES AND EXCRETIONS OF
IRON, COPPER, AND ZINC IN THE NEONATAL PERIOD

BY

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Rapid alterations in the distribution of iron, copper, and zinc within the body take place in the neonatal period, and the direction of the change is sometimes a reversal of that which has been going on during foetal life. Iron has attracted most attention because the fall in circulating haemoglobin that takes place in the weeks after birth can be measured comparatively easily. From time to time during the present century, however, investigators have measured the intakes and excretions of iron by babies in the first 6 months of life, often with the object of tracing the fate of the iron set free by the breakdown of red cells. Some of the infants have been breast fed, and others have received mixtures made up from cows' or goats' milk; some have received supplements of one sort or another while others have had nothing but the milk in question. The results have been conflicting from first to last, even among investigators who have studied babies receiving breast milk and nothing else; no correlation has been found between the amount of iron set free from haemoglobin during the period of red cell destruction and the iron balance, and this cannot be attributed to faulty technique. The age and health of the baby and whether it was born prematurely or at term may be important. Langstein and Edelstein (1914) followed one breast-fed baby through the first 12 days after birth and found that it was in negative balance for the first 7 days and in positive balance for the next 5. Two other breast-fed babies aged 8 and 12 weeks were both in positive balance. Lichtenstein (1921) studied 4 premature babies receiving breast milk between the time they were 3 to 4 weeks and 4 months old. All the babies were in negative iron balance on every occasion. Wallgren (1933) had 5 breast-fed babies in his series. They were all studied on more than one occasion, the youngest age being 3 weeks, and the oldest 11 months: 3 babies were always in positive balance while the balances of the other 2 were sometimes negative, sometimes positive. Josephs (1934) measured the intakes and excretions of iron by 6 babies, but only 1 was breast fed: this baby retained a small amount of iron from the third to the fifth week but had a large negative balance over the next two months. Similarly, of the 14 infants studied by Stearns and Stinger (1937) only 1 was given human milk. At 9 and 13 weeks the excretion was equal to the intake. Maurer, Greengard, Curtis, and Klüver (1934) reported that 5 breast-fed infants aged 3 weeks to 3 months absorbed and retained 78-100% of their iron intake; they and others (Stearns and McKinley, 1937) agree that babies having cows' milk preparations are more likely to be in negative iron balance than those that are breast fed.

The newborn period is the time of postnatal life when the anabolic tendency is at its greatest, and the young baby retains a higher proportion of most of the physiological constituents of its food than it is ever likely to do again (Slater, 1961). If babies really are frequently in negative iron balance the fact must be accepted, curious though it may seem, but little work has been done on the subject in recent years and a further investigation with rigid control seemed highly desirable.

The present study has been limited to the neonatal period, that is, to babies during the first 8 days after birth, and has embraced copper and zinc as well as iron. Only one study of copper metabolism at this age has been found (Kleinbaum 1962) and, apart from Berfenstam's (1952) report of the intake and faecal excretion of zinc by three babies with meningomyelocele, there seemed to be no information whatever about the baby's metabolism of zinc.

Subjects and Methods

Subjects. All babies studied in this investigation were born at the Cambridge Maternity Hospital. Meconium was collected for the first 24 hours after birth from 5 full-term girls and 1 boy. There were 10 normal full-term boys who were the subjects for the mineral balances, which were made at the end of the first week.

These 10 babies were all doing well on breast milk and had received no supplement of any kind.

Meconium. Meconium was collected on weighed napkins made of polyethylene, which were changed as soon as possible after passage of a stool, and there was never any evidence that this was contaminated with urine. The napkins containing the stool were weighed and then washed with hot de-ionized water. The washings were carefully dried and the solids ground as finely as possible, avoiding mineral contamination. The total solids were weighed and duplicate samples (1-1·5 g.) were ashed in silica crucibles at 450° C. for mineral analyses.

Mineral Balances. These studies usually were made from the 6th to the 8th day after birth. For three babies the time ran from the 5th to the 7th day, and for one baby only a 2-day balance was possible, on the 6th and 7th days. The procedures followed for the collection of the samples were, with a few modifications, those outlined by Widdowson, Cavell, and McKeag (1964). All samples were collected and stored in polyethylene bottles. De-ionized water was used for rinsing when necessary. The used napkins were washed as described (Widdowson et al., 1964), but de-ionized water and HCl were used. A sample of the acid solution used was kept as a blank on the washing procedure. The faeces and washings were homogenized by prolonged mechanical shaking in polyethylene containers, pooled, the total volume registered, and a measured portion of about one-tenth was taken for analysis.

The pooled volumes of milk and of urine were sampled as described by Widdowson et al. (1964), but 0·5 ml. HNO_3 was added to each sample before ashing. All samples were carefully ashed in silica crucibles at 450° C.

Blanks on the reagents were regularly carried out; the one for the milk and urine consisted of 0·5 ml. HNO_3, and the one for the faeces of a volume of HCl solution equal to the volume taken for analysis.

Chemical Analysis. After they had been ashed the samples of meconium, milk, urine, and faeces were treated alike. The ash was extracted by the method outlined by McCance and Shipp (1933), but the filter paper was first washed with hot 50% ($\frac{v}{v}$) HCl followed by de-ionized water, and the extraction was made with 1 ml. conc. HCl and 20 ml. N/2 HCl. A similar procedure was followed with the residues from the two blanks from which the acid had been evaporated. De-ionized water was used throughout. The analyses were made on these ash extracts and all the analyses for an individual baby's balance study were carried out in one batch. Glassware, mainly pyrex to avoid contamination, was carefully washed with chromic acid and rinsed finally with de-ionized water. All pipettes and, as far as possible, other glassware were used only for the trace metal work. Silica crucibles were cleaned with dilute nitric acid before each use.

Iron was determined by the standard o-phenanthroline method as outlined by Sandell (1944). Copper was determined colorimetrically by the diethyldithio-carbamate method in which the colour was extracted from ammoniacal solution with amyl alcohol. The alcohol was siphoned off, dried by mixing with anhydrous sodium sulphate, and cleared by centrifuging. The colour was read at 435 μ against alcohol and compared with standards carried through the procedure. This basic method was that of Eden and Green (1940). Zinc was determined as the dithizonate by the mixed colour method outlined by Sandell (1944). A set of standards was carried through this procedure for each balance.

Results

Excretion of Iron, Copper, and Zinc in Meconium During First 24 Hours After Birth. Table 1 shows the concentrations of iron, copper, and zinc in meconium passed by six babies in the first 24 hours after birth. The mean concentrations of iron and copper were approximately equal, that of zinc 4 times as high. The Table also shows the total excretions of the three elements. These values partly depend upon the amount of meconium the babies happened to pass in the first 24 hours, which varied from 20·7 to 47·7 g. They do not represent the total amounts of the elements in the whole of the meconium filling the large intestine at the time of birth.

Iron, Copper, and Zinc in Breast Milk. Table 2 shows the concentrations of iron, copper, and zinc in the breast milk from the mothers whose babies were studied when they were 1 week old. It also gives the age of the baby, the mean weight, and the

TABLE 1
COMPOSITION AND EXCRETION OF MECONIUM IN THE FIRST 24 HOURS AFTER BIRTH

Baby No.	Birth Weight (kg.)	Composition of Meconium (mg./100 g. wet weight)			Excretion in Meconium During First 24 hr. (mg./kg. body weight)		
		Iron	Copper	Zinc	Iron	Copper	Zinc
1	3·65	1·67	1·70	10·02	0·16	0·16	0·95
2	3·37	2·71	2·47	11·73	0·17	0·15	1·08
3	2·94	1·75	1·53	3·63	0·15	0·13	0·31
4	3·74	1·20	1·75	4·16	0·13	0·19	0·44
5	3·65	1·45	1·80	3·88	0·19	0·24	0·51
6	3·12	1·31	0·95	5·57	0·16	0·12	0·69
Mean	3·41	1·68	1·70	6·50	0·16	0·17	0·66

TABLE 2

DESCRIPTION OF BABIES AND COMPOSITION OF BREAST MILK

Baby No.	Age During Balance Period (days)	Mean Weight During Balance Period (kg.)	Mean Milk Intake During Balance Period (ml. kg. day)	Composition of Breast Milk (mg./100 ml.)		
				Iron	Copper	Zinc
7	5–7	4·13	142	0·033	0·066	0·142
8	5–7	2·63	156	0·052	0·052	0·168
9	5–7	3·12	113	0·039	0·058	0·343
10	6–7	4·04	175	0·100	0·059	0·645
11	6–8	4·27	176	0·040	0·071	0·677
12	6–8	3·22	207	0·145	0·077	0·573
13	6–8	3·10	137	0·060	0·061	0·506
14	6–8	3·24	170	0·050	0·051	0·157
15	6–8	4·47	174	0·029	0·055	0·265
16	6–8	3·59	148	0·041	0·067	0·600
Mean		3·58	160	0·059	0·062	0·408

TABLE 3

IRON BALANCES (mg. kg. day)

Baby No.	Intake	Excretion			'Absorption' (Food − Faeces)	Balance
		Faeces	Urine	Total		
7	0·047	0·568	0·001	0·569	−0·521	−0·522
8	0·080	2·422	0·001	2·423	−2·342	−2·343
9	0·044	0·457	0·002	0·459	−0·413	−0·415
10	0·176	0·758	0·002	0·760	−0·582	−0·584
11	0·070	1·071	0·001	1·072	−1·001	−1·002
12	0·302	1·165	0·003	1·168	−0·863	−0·866
13	0·082	1·188	0·001	1·189	−1·106	−1·107
14	0·085	1·760	0·001	1·761	−1·675	−1·676
15	0·050	1·357	0·003	1·360	−1·307	−1·310
16	0·061	0·477	0·002	0·479	−0·416	−0·418
Mean	0·100	1·122	0·002	1·124	−1·022	−1·024

mean intake of milk over the balance period. The concentrations of iron and zinc varied more than that of copper, and there was little correlation between the concentrations of the three elements. The mean concentrations of iron and copper were similar to each other, and that of zinc four times as great. Feuillen and Plumier (1952) analysed a number of samples of breast milk for iron, and their range of values agrees with ours. The concentration of zinc is much higher in colostrum than in mature breast milk (Berfenstam, 1952); our values for the 5th to 8th day are in the same range as those of Berfenstam. Our mean values for all three metals are similar to those given by Macy, Kelly, and Sloan (1953), who also found, as we have, considerable variations between milk from different mothers. A high intake of milk has not necessarily coincided with low concentrations of the trace elements in it. Baby 12 in our series, for example, took twice as much milk per kg. of body weight as Baby 9, and his mother's milk had higher concentrations of iron, copper, and zinc. The intakes of the metals by the different babies during the balance period varied even more than either the concentrations of the elements in the milk or the volumes of milk taken by the babies.

Iron Balances. All the babies were in negative iron balance and the faeces contained 4 to 30 times as

much as the food (Table 3). Only traces were excreted in the urine, and in most previous studies of iron balance in early life the urine has not even been collected, or has been included with the faeces. The average loss of iron from the body was just over 1 mg. /kg. day or 3·5 mg. day for an average baby. The excretion of iron in the faeces bore no relation to the amount in the food, and most of the faecal iron was clearly endogenous in origin. At the age of 1 week these babies were excreting about 7 times as much iron per day in the faeces as Babies 1-6 lost in the meconium during the first day after birth.

Copper Balances. Table 4 shows the intakes and excretions of copper by 10 babies 1 week old: 3 were in positive, and 5 in negative balance, and the total excretion was approximately equal to the intake in 2. These results were similar to those of Kleinbaum (1962). Of his 6 full-term babies, 13 days old, 3 were in positive, and 3 in negative balance. The amount of copper in the urine, as of iron, was negligible and negative balances were always due to high faecal excretion. There was some relation between the amount of copper taken in the milk and the amount excreted in the faeces, and the highest intakes were associated with the greatest excretions. The daily quantities of copper in the faeces at 1 week of age were less than those in the meconium on the

TABLE 4

COPPER BALANCES (mg./kg. day)

Baby No.	Intake	Excretion			'Absorption' (Food − Faeces)	Balance
		Faeces	Urine	Total		
7	0·095	0·073	0·003	0·076	−0·022	+0·019
8	0·081	0·083	trace	0·083	−0·002	−0·002
9	0·065	0·046	trace	0·046	+0·019	+0·019
10	0·104	0·075	nil	0·075	+0·029	+0·029
11	0·126	0·124	0·002	0·126	+0·002	±0
12	0·159	0·197	0·003	0·200	−0·038	−0·041
13	0·083	0·119	0·001	0·120	−0·036	−0·037
14	0·088	0·153	0·001	0·154	−0·065	−0·066
15	0·096	0·103	0·003	0·106	−0·007	−0·010
16	0·099	0·097	0·002	0·099	+0·002	±0
Mean	0·100	0·107	0·002	0·109	−0·007	−0·009

TABLE 5

ZINC BALANCES (mg./kg. day)

Baby No.	Intake	Excretion			'Absorption' (Food − Faeces)	Balance
		Faeces	Urine	Total		
7	0·20	0·41	0·05	0·46	−0·21	−0·26
8	0·26	0·50	0·03	0·53	−0·24	−0·27
9	0·39	0·37	0·12	0·49	+0·02	−0·10
10	1·14	1·30	0·06	1·36	−0·18	−0·22
11	1·19	1·04	0·07	1·11	−0·15	+0·08
12	1·19	1·32	0·06	1·38	−0·13	−0·19
13	0·69	0·76	0·09	0·85	−0·07	−0·16
14	0·27	1·09	0·03	1·12	−0·82	−0·85
15	0·46	0·57	0·08	0·65	−0·11	−0·19
16	0·89	0·89	0·06	0·95	±0	−0·06
Mean	0·67	0·83	0·07	0·90	−0·16	−0·23

first day. The iron/copper ratios in meconium and in the faeces at 1 week of age were, therefore, very different, being about 1 and 10, respectively.

Zinc Balances. The results for the zinc balances are given in Table 5. All but one were in negative balance. Most of the zinc was being excreted by the intestine and 7 of the babies had more in their faeces than in their food. The daily excretion of zinc by the bowel at 1 week of age was about the same as that during the first day. In all but one baby there was a relation between intake of zinc in the milk and its excretion in the faeces. About 8°₀ of the total zinc excreted was found in the urine, and the mean concentration in infant's urine (0·1 mg./100 ml.) was about 5 times as high as that in adult urine (McCance and Widdowson, 1942).

Discussion

The outstanding question raised by the results of this study is how and why do babies at the end of the first week after birth excrete more iron and zinc in their stools than they get from their milk, and sometimes more copper as well? The quantities involved are considerable. Table 6 shows the approximate amounts of iron, copper, and zinc in the body of a baby weighing 3·5 kg. (Widdowson and Spray, 1951; Widdowson and Dickerson, 1964), the mean daily intake of the three elements as found in this study, and the mean daily losses from the body. The baby gets from breast milk every day amounts equivalent to about 0·1°₀ of its body iron, 2·5°₀ of its copper, and 4·3°₀ of its zinc. The losses correspond to more than 1°₀ of the body's iron and zinc and 0·2°₀ of the copper. There is no evidence from the results of

TABLE 6

COMPARISON OF DAILY INTAKES AND LOSSES OF IRON. COPPER. AND ZINC WITH TOTAL AMOUNTS IN BODY

	Fe	Cu	Zn
Mean amount in body of 3·5 kg. baby (mg.)	320	13·7	53
Mean intake at 6–8 days of age (mg. day)	0·35	0·35	2·3
Mean loss from body (mg. day)	3·6	0·04	0·80

this investigation how long these losses continue, though they certainly continued for the 3 days of the study. On the face of it, it seems that losses as large as this must be a temporary phenomenon, peculiar to the neonatal period, for if this were not so the body would soon become seriously depleted. Haemolysed red blood cells are the probable source of the iron, and also of the zinc (Berfenstam 1952), but when iron is set free inside the body of an adult by the breakdown of red cells due to the administration of phenylhydrazine (McCance and Widdowson, 1937a) or in patients suffering from severe haemolytic anaemia (McCance and Widdowson, 1943; Granick, 1949), no significant amount of the iron is excreted. Indeed, iron once incorporated into the body of an adult is not excreted by the intestine in more than traces, and the amount of iron in the body is regulated by controlled absorption (McCance and Widdowson, 1937b; Maddock and Heath, 1939). The mechanism by which absorption is controlled is still not clearly understood (*Lancet*, 1963), but there seems no doubt that when erythropoietic activity is high iron absorption is increased, and conversely when it is low iron absorption is diminished (Bethard, 1962). In neither situation, however, do the faeces contain more iron than the food. This is evidently not the case in young babies, though why the iron and indeed the zinc and copper enter the intestine is not known. The bile is a possible source of the iron and copper, for Sheldon and Ramage (1931, 1933) using a spectrographic method found foetal bile to contain remarkably high concentrations of the two metals, and this may be the origin of some of the iron and copper in the meconium. Bile secreted by adults normally contains very little iron, but the concentration may increase during periods of intense haemolysis (Hawkins and Hahn, 1944). In the adult the iron reaching the intestine in the bile, or an equivalent amount derived from the food, must be reabsorbed if the person is to remain in iron balance even when there is extensive destruction of red cells going on inside the body. Josephs (1953) suggested that iron as it appears in the bile may be much more efficiently absorbed than other forms of the metal. Whether the bile of the young baby contains a particularly large amount of iron or whether the baby is less able than the adult to reabsorb this iron, or whether some other intestinal secretion is a more important source of the iron in the infant's faeces still remains to be discovered.

Little may be known about the fundamental aspects of iron metabolism in the neonatal period, but still less information is available about the metabolism of copper and zinc, and speculation about it would be futile. Whether the loss of iron, copper, and zinc from the body in the neonatal period is peculiar to the human species, or whether it is true of other mammals is also not yet known. That it may be a more general phenomenon is suggested by the observations of Bertrand and Vladesco (1921) and Lintzel, Rechenberger, and Schairer (1944) who found that the amount of zinc and of iron in the body of the rabbit fell during the first few days after birth, and the finding of Manners and McCrea (1963) that pigs, 2 days old, contained less iron in their bodies than litter-mates at the time of birth.

Summary

Meconium passed during the first 24 hours after birth was collected from 6 babies and analysed for iron, copper, and zinc. The intake and urinary and faecal excretion of the three metals were measured on another 10 babies, all breast-fed, over a three-day period at the end of the first week.

Meconium contained an average of 1·7 mg./100 g. iron and copper and 6·5 mg./100 g. zinc.

The concentration of all three metals was very variable in breast milk and there was no relation between the volume of milk secreted and its composition.

All the babies were in negative iron balance; the faeces contained on the average more than 10 times as much iron as the food and the mean loss of iron from the baby's body amounted to 3·6 mg. a day.

Some of the babies were in negative and some in positive copper balance, but all except one were in negative zinc balance. They were losing more than 1% of the body's total iron and zinc each day. It is not known how long these large negative balances continue, but possible explanations for them are discussed.

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