Proc. Nutr. Soc. (1974), **33**, 275

Trace elements in foetal and early postnatal development

By ELSIE M. WIDDOWSON, *Department of Investigative Medicine, Downing Street, Cambridge CB2 1QN*, JOY DAUNCEY, *Dunn Nutritional Laboratory, Cambridge CB4 1XJ* and J. C. L. SHAW, *Department of Paediatrics, University College Hospital, London*

This paper will deal mainly with three trace elements, zinc, copper and iron. I shall first discuss their transfer to the foetus, their combination and concentration in the serum on both sides of the placenta and their accumulation in the foetal body. I shall then pass on to the requirements and intakes of the full-term infant during the period after birth while it is living on milk, and finally show some of the problems facing the pre-term, low-birth-weight baby if it is to acquire as much Zn, Cu and Fe as it would have laid down in its body had it not been born.

Foetal development

Trace elements must enter the body of the foetus from very early in gestation. In order to do this they must cross the placenta, but how they achieve this is not entirely clear. Many of them circulate in the serum combined with protein, and these metal–protein complexes are unlikely to pass the placental barrier as such; they must be broken down and the metal combined in a more soluble form, possibly with amino acids, and then become recomplexed with protein again in the foetal serum.

Zn. Part of the Zn in the serum is loosely associated with serum albumin, and part more firmly bound to globulin. The concentration of Zn in the mother's serum falls during pregnancy (Table 1). The concentration in the serum of a 20–26-week-old foetus is very high and seems to fall towards term, but the serum of the baby has a higher concentration than that of its mother whether birth has been premature or not (Berfenstam, 1949, 1952; Vikbladh, 1951). It is important to take into account those physiological variations in any consideration of Zn deficiency based on a low level of Zn in the serum.

About 90% of the Zn in the blood of an adult is in the erythrocytes, mainly as a constituent of the enzyme carbonic anhydrase (*EC* 4.2.1.1). There is only one-quarter as much Zn in the erythrocytes of the foetus and baby at term as there is in those of the adult, which reflects their low levels of carbonic anhydrase (Stevenson, 1943). The concentration of Zn in the erythrocytes of the foetus is in fact no higher than the concentration in its serum.

Fig. 1 shows the total amount of Zn in the developing human foetus, and the quantity of it that is present in the liver (Widdowson & Spray, 1951; Widdowson, Chan, Harrison & Milner, 1972). The liver accounts for about one-quarter of the

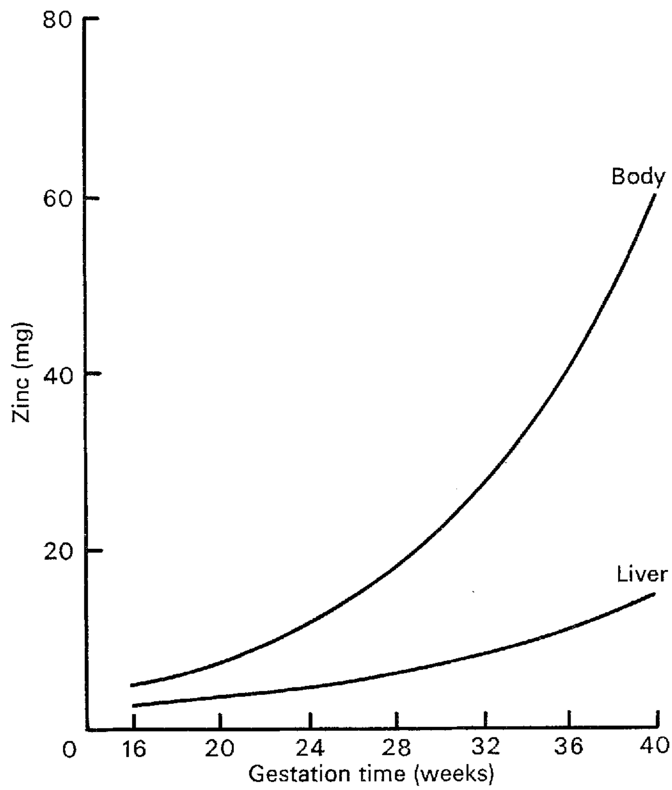

Fig. 1. Accumulation of zinc in the body and liver of the developing human foetus, fitted to the equation $y = y_0 e^{Kt}$: for the body, $\log_{10} y_0$ (mg) $= \bar{1}{\cdot}9635$, $\log_{10}$ K (t in weeks) $= 0{\cdot}0454$, $r = 0{\cdot}94$; for the liver, $\log_{10} y_0$ (mg) $= 0{\cdot}0087$, $\log_{10}$ K $= 0{\cdot}0289$, $r = 0{\cdot}78$.

Zn in the human body at term. Most of the Zn in the liver is bound to proteins, part as metallo-enzymes, and part more loosely bound. Other quantitatively important sites in the body where Zn collects are the bones, and the hair, fur or wool, and animals that are born covered with hair, like kittens and guinea-pigs, have higher over-all concentrations of Zn in their bodies than the human baby (0·29 and 0·35 mg/g compared with 0·19 mg/g) (Spray & Widdowson, 1950).

Cu. Cu in the tissues and blood forms stable complexes and chelates with many organic molecules. In the serum most of it is present as part of the enzyme caeruloplasmin, but some is attached to albumin and amino acids in a more loosely bound form. The concentration of Cu in the serum, shown in Table 1, is lower in the infant at term (500 μg/l) than it will be at any other time of its life. The concentration, however, is particularly high on the maternal side of the placenta, and the value for the mother's serum is about five times higher than that for the newborn infant's (Scheinberg, Cook & Murphy, 1954). The serum Cu concentration of a woman rises during pregnancy (Krebs, 1928; Fay, Cartwright & Wintrobe, 1949; Markowitz, Gubler, Mahoney, Cartwright & Wintrobe, 1955; Adelstein, Coombs & Vallee, 1956), and the concentration of Cu in maternal serum at term is about

Table 1. *Zinc, copper and iron concentrations in human serum* ($\mu g/l$)

	Foetus		Mother at term	Non-pregnant woman
	Immature	Full-term		
Zn	3000	1250	700	1200
Cu	2000	500	2500	1000
Fe	400	1600	600	700–1500

2·5 mg/l compared with 1·0 mg/l for a non-pregnant woman. The changes in serum Cu, therefore, are in the opposite direction to those for serum Zn. The difference between the serum Cu concentrations of mother and baby is made possible by the high concentration of caeruloplasmin in the maternal serum, and the concentration of Cu not in this form is the same on both sides of the placenta (Scheinberg *et al.* 1954). Presumably only the non-caeruloplasmin fraction passes over to the foetus, and it is not until after birth that the baby acquires the ability to synthesize caeruloplasmin for itself.

The ability of the foetal liver to accumulate Cu has intrigued investigators since the study of the distribution and function of this element began. The livers of the foetal rat, rabbit, guinea-pig, dog and pig all have high concentrations of Cu (Cunningham, 1931; McFarlane & Milne, 1934; Wilkerson, 1934; Brückmann & Zondek, 1940). Fig. 2 shows that this is also true of man. About half the Cu in the body of the human foetus is in its liver. Much of the Cu in foetal liver is in the mitochondria as a Cu–protein complex which Porter (1966) named 'neonatal hepatic mitochondrocuprein'. This complex is peculiar to the foetus, and contains ten times as much Cu as any other known protein. It is believed to be a storage compound, not an enzyme. It begins to disappear soon after birth, and the Cu set free from it is presumably used for the needs of other tissues of the growing body. Since it is in the liver that Cu is incorporated into caeruloplasmin, it seems likely that some of the Cu set free is used for this purpose.

Fe. The 'average' term baby is born with about 300 mg Fe in its body. Most of this is in protein-bound complexes, and 60–70% of it is present as part of the haemoglobin molecule. Fe in serum is completely non-dialysable, and most of it is attached to the protein which transports Fe, transferrin. In spite of the way in which it is combined in the serum, Fe seems to move freely from the mother's serum to the foetus in man (Pommerenke, Hahn, Bale & Balfour, 1942), the guinea-pig (Vosburgh & Flexner, 1950) and the rabbit (Bothwell, Pribilla, Mebust & Finch, 1958). It seems, too, that once it has reached the foetus it remains there. The concentration of Fe in human foetal serum rises during the last 3 months of gestation to about 1·6 mg/l (Vahlquist, 1941), which is about three times the concentration in the serum of the mother.

Fig 3. shows the amount of Fe in the growing body of the foetus, and the amount of Fe in its liver. Ferritin and haemosiderin are the main chemical forms in which Fe is stored, and it is mobilized from both sources when the need arises (Shoden, Gabrio & Finch, 1953). Although a smaller proportion of the body's Fe than of its

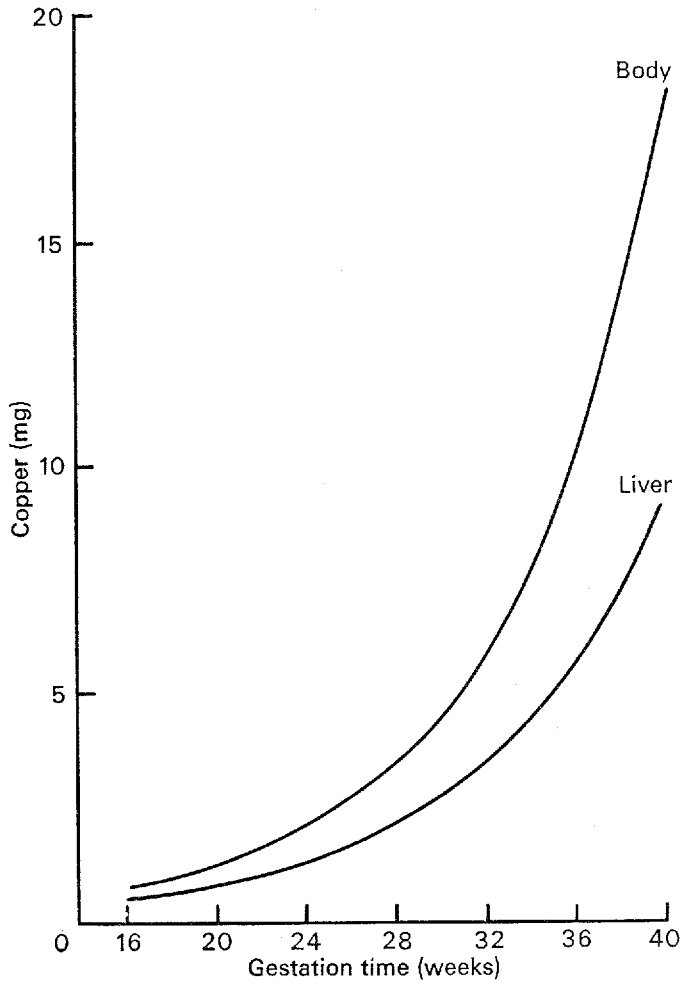

Fig. 2. Accumulation of copper in the body and liver of the developing human foetus, fitted to the equation $y = y_0 e^{Kt}$: for the body, $\log_{10} y_0$ (mg)$=\bar{2}\cdot9032$, $\log_{10}$ K (t in weeks)$=0\cdot0586$, $r=0\cdot97$; for the liver, $\log_{10} y_0$ (mg)$=\bar{2}\cdot8442$, $\log_{10}$ K$=0\cdot0529$, $r=0\cdot93$.

Zn or Cu is present in the foetal liver, the pre-term infant, as Fig. 3 shows, is at a disadvantage so far as its Fe reserves are concerned. Chang (1973) analysed the livers from twelve premature and twelve mature but still-born babies and found that the Fe content of the liver more than doubled during the last weeks of gestation. His values are in line with those shown in Fig. 3. However, even in the infant at term the 50 mg Fe in its liver would not go far towards providing the amount it will require to maintain the concentration of Fe in its body while it is living on milk.

Other trace elements. There are no values for the amounts of other trace elements in the whole body of the developing human foetus, but livers have been analysed for manganese (Widdowson *et al.* 1972). The foetal liver does not appear to 'store' Mn as it does Cu, and to a lesser extent Zn and Fe, yet the concentration in milk is

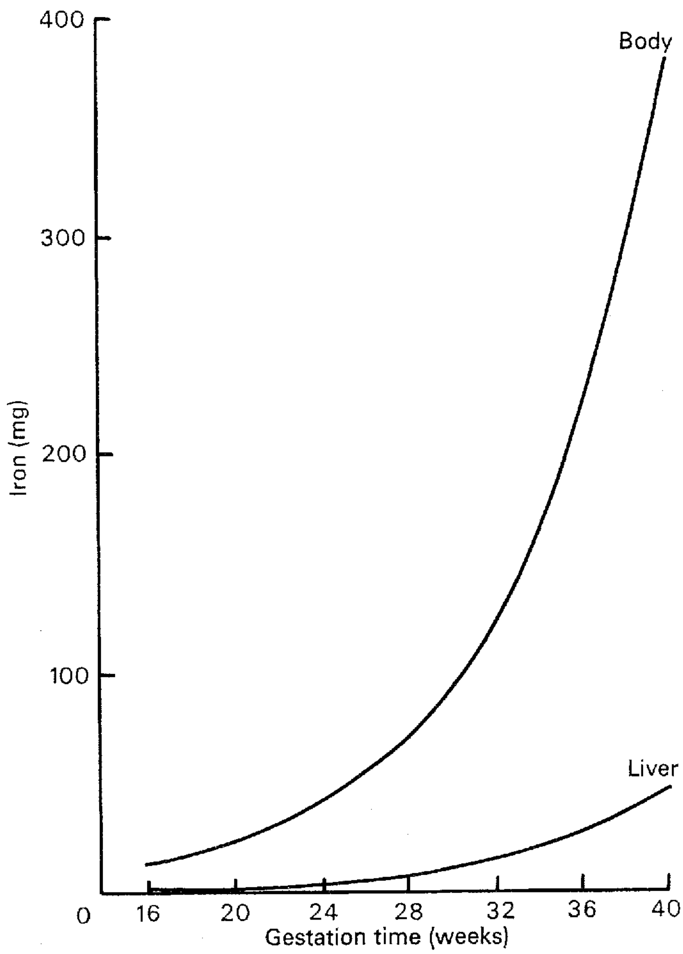

Fig. 3. Accumulation of iron in the body and liver of the developing human foetus, fitted to the equation $y=y_0\ e^{Kt}$: for the body, $\log_{10} y_0$ (mg)=0·1280, $\log_{10}$ K (t in weeks)=0·0613, r=0·96; for the liver, $\log_{10} y_0$ (mg)=$\bar{1}$·0569, $\log_{10}$ K=0·0654, r=0·87.

very low. It is puzzling how the baby obtains enough Mn for its needs after birth, but it is possible that the bones constitute the foetal 'store'. The ribs of foetal pigs were found to have a much higher concentration of Mn than other foetal tissues and than the same part of the rib of the mother (Gamble, Hansard, Moss, Davis & Lidvall, 1971). Mn deficiency has been shown to prevent the normal development of the epiphyseal cartilages of the bones in foetal guinea-pigs (Tsai & Everson, 1967) and of the otoliths of the inner ear (Shrader & Everson, 1967).

Requirements and intakes of infants born at term

Table 2 shows the concentration of Zn, Cu, Fe and Mn in human and cow's milk. The concentration of Zn in both falls during lactation from over 20 mg/l in colostrum

Table 2. *Concentrations of zinc, copper, iron and manganese in mature human and cow's milk* (μg/l)

	Human	Cow's
Zn	3000–4000	3000–4000
Cu	150–170	50–150
Fe	300–600	300–600
Mn	7	20–30

to 3–4 mg/l after several months (Berfenstam, 1952). Both milks provide enough Zn for the full-term baby if about 15% of the intake is absorbed and retained.

Values given for the concentration of Cu in milk vary considerably from one analyst to another, but all agree that it is very low, and that cow's milk contains less than human milk. The full-term baby would need considerably more than the amounts provided by either milk to maintain the concentration of Cu in its body, even if most of the Cu in the milk were absorbed. Whether this is necessary or not in view of the large store in the liver is another question. It is probable that Cu is absorbed more efficiently from human than from cow's milk because of the known interaction between Cu and Zn. The Zn:Cu ratio in human milk is about 4, but that in cow's milk is higher. A high Zn:Cu ratio is known to depress the absorption of Cu, and Cu deficiency has been reported in a premature infant fed on a preparation based on cow's milk (Al-Rashid & Spangler, 1971).

It has been known for many years that milk contains far too little Fe for the growing baby if the concentration of haemoglobin in its blood is to be maintained, even at the level reached after the immediate postnatal fall. Fomon (1967) calculated that the increment of Fe in the body over the first year should be about 200 mg, of which 100 mg should be retained during the first 6 months. If this is so, then the average daily increment should be 0·55 mg. The Fe intake of a baby living on human or unfortified cow's milk would be about 0·33 mg/d, varying with the age of the baby and the volume of milk taken. The baby will not absorb the whole of its intake, and balance studies with ^{59}Fe, where the infants were given milk from a cow that had been infused with the isotope, suggest that absorption of 10% of the intake is a reasonable estimate after 3 months of age (Schulz & Smith, 1958). Thus an average daily intake of 5·5 mg assimilable Fe ought to cover most babies' requirements. Manufacturers of many infant foods add an Fe salt to their preparations, and the daily intakes of Fe from these would vary from a little over 4 mg, for Cow and Gate Babymilks 1 & 2 (Unigate Foods Ltd, Guildford) and National Dried Milk (Welfare Foods), to 8–10 mg for Ostermilk (Glaxo Ltd, Greenford, Middx) and SMA (John Wyeth Ltd, Maidenhead).

Special problems of low-birth-weight infants

Since about two-thirds of the Zn, Cu and Fe in the foetal body at term are transferred to it during the last 10–12 weeks of gestation, infants born at 28–30 weeks gestation, weighing only about 1 kg, are faced with very serious problems.

Even if precautions are taken to ensure that their food contains enough, they are unlikely to absorb a high proportion of what they take in, and a high intake of one element may interfere with the absorption of another. In order to find out how much of the Zn, Cu and Fe low-birth-weight babies absorbed and retained, balance studies have been carried out continuously for 3–6 weeks on six such infants who weighed less than 1·5 kg at birth. Two were born after 37 and 38 weeks gestation and, since they weighed only a little over 1 kg, they were very small indeed for their gestational age. They received human milk by bottle and the balance studies started when they were 10 and 15 d old. The other four were born after 28–31 weeks gestation; their weights were appropriate for their gestational ages, and they were developmentally less mature than the first two. Two of these premature babies had feeds based on cow's milk: one had a half-skimmed dried milk preparation, and she was first studied at 15 d old. The other had full-cream evaporated milk and he was 20 d old when the study began. The last two had 'Ready to Feed' SMA. This is a milk preparation in which all the cow's milk fat is replaced by a mixture of animal and vegetable fats. It contains added Cu and Zn. The balance study on one of these babies did not begin until she was 35 d old. The study on the other began at 15 d.

Fig. 4 shows the retentions of Zn, Cu and Fe by the four pre-term babies compared with the average increments in the body of a foetus growing over the same

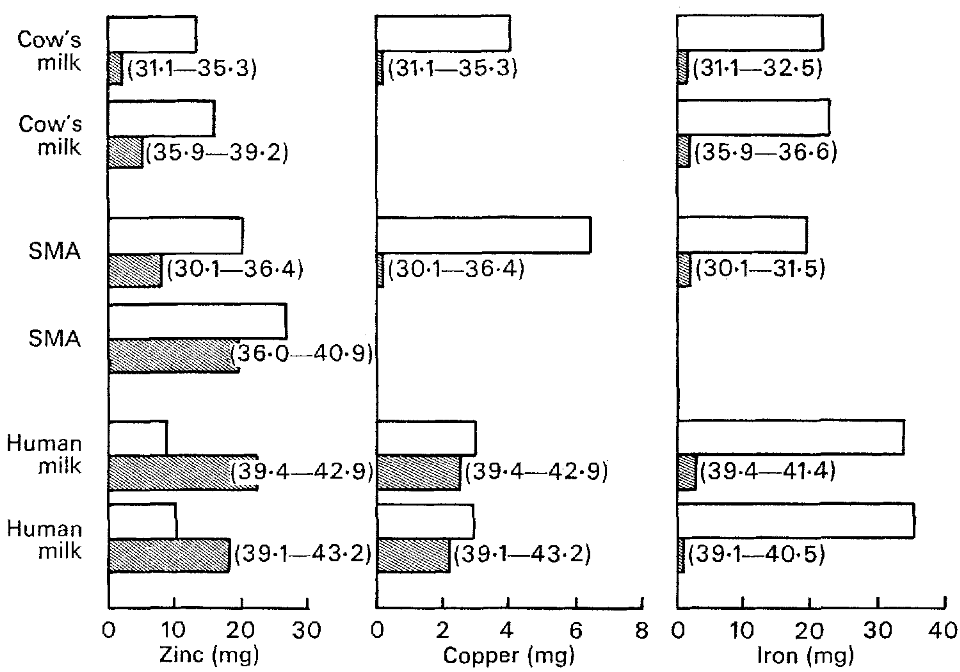

Fig. 4. Retentions of zinc, copper and iron by six low-birth-weight babies given different milks, compared with retentions in utero. □, Retention in utero; ▨, retention after birth. Gestational age (weeks) during study is shown in parentheses. SMA, proprietary artificial milk (John Wyeth Ltd, Maidenhead).

period in utero: the two small full-term infants cannot be compared on this basis and the amount of each element retained by them has been compared with the increments in a foetus over an equivalent weight gain. Neither of the babies having cow's milk preparations retained as much Zn as an unborn foetus. Their intakes of Zn were quite high, but they absorbed only 15% and 30% of this over the period as a whole, and they were both in negative balance during the first week or so. The same was true of one of the babies having SMA. Full-term, breast-fed babies have previously been found to be in negative Zn balance at the end of the first week after birth (Cavell & Widdowson, 1964). None of the present balance studies started until at least 15 d after birth, and we suspect that the babies may have been in negative balance during these first 15 d also. In fact, the two babies having cow's milk preparations and one having SMA did not begin to retain Zn until 25 d after birth. The other baby having SMA retained considerably more Zn. However, balance measurements were not begun until she was 35 d old and, although she was in positive balance throughout the period of observation, she may well have been in negative Zn balance in her early days as the other babies were. If this was so, she may have had a rather larger body deficit of Zn at the end of the study than is suggested by these results.

The two full-term babies given human milk appear to have retained Zn well when compared with a foetus of equivalent weight. However, we do not know how much Zn there was in the bodies of these small full-term babies at the start and, moreover, they were given more food than the others and they were growing very rapidly indeed.

The results for Cu show that the two full-term babies having human milk made a better showing than the pre-term ones given cow's milk or SMA. Although SMA contains enough added Cu to make the concentration similar to that in human milk, the baby receiving it retained virtually none, and did no better than the baby having cow's milk with less than half as much Cu. If these two are representative of other pre-term, low-birth-weight babies, it looks as though their bodies, and particularly their livers, will be seriously deficient in Cu if they have foods based on cow's milk. Their poor absorptions and retentions of Cu may be related to their comparatively high intakes of Zn.

Measurements of Fe absorption were made on five of the babies during the first week, but stopped when Fe supplementation began since the supplements had not been measured accurately. Fig. 4 shows that none of the babies retained any significant amount of Fe during the first period. Two other pre-term babies, one weighing 1·2 kg and having breast milk, the other weighing 1·1 kg and having SMA, have been studied over 2–3 weeks while they were being given additional Fe, between 8 and 10 mg/d, with 21 mg/d for the second baby over a short period. The cumulative retentions of Fe by these two babies are shown in Fig. 5. Although both were in positive balance and retained 20–25% of all the Fe they were given, neither absorbed or retained as much as they would have added to their bodies if they had been growing over the same gestational age in utero.

It has been shown previously that low-birth-weight babies do not retain enough

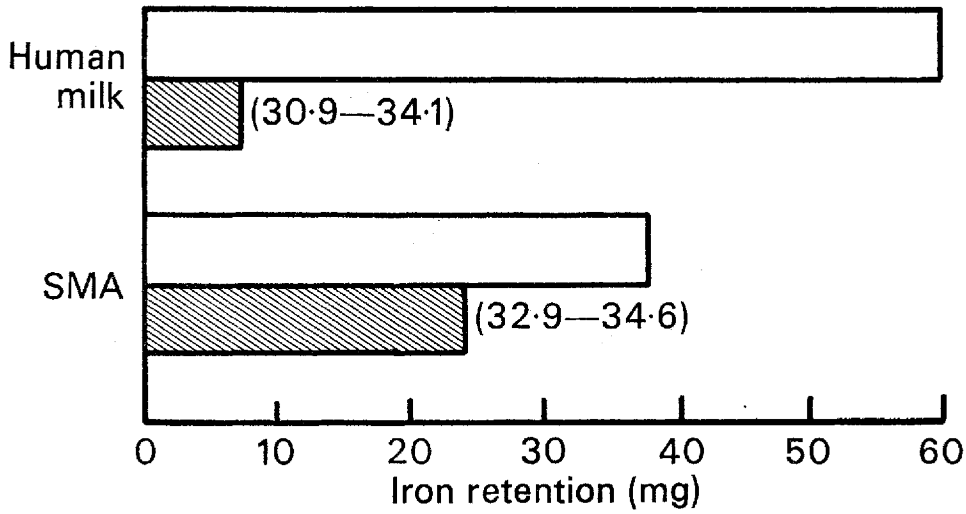

Fig. 5. Retentions of iron by two low-birth-weight babies who were given supplementary Fe, compared with retentions in utero. □, Retention in utero; ▨, retention after birth. Gestational age (weeks) during study is shown in parentheses. SMA, proprietary artificial milk (John Wyeth Ltd, Maidenhead).

calcium for their needs (Shaw, 1973). These results on trace elements are preliminary, but they suggest that small pre-term babies are likely to have less Zn, Cu and Fe in their bodies when they reach a gestational age of 40 weeks than a full-term baby at birth. Whether they are any the worse clinically for this we do not know, for such babies have not been examined carefully over a longer period with this in mind. We know that they grow slowly, but whether this has anything to do with trace element deficiencies is still an open question. All the same, there is a strong case for trying to design a special milk for such babies which will at least give them a chance of retaining an average of 300 μg Zn, 85 μg Cu and 1·8 mg Fe/kg body-weight per d.

REFERENCES

Adelstein, S. J., Coombs, T. L. & Vallee, B. L. (1956). *New Engl. J. Med.* **255**, 105.
Al-Rashid, R. A. & Spangler, J. (1971). *New Engl. J. Med.* **285**, 841.
Berfenstam, R. (1949). *Acta paediat., Stockh.* **37**, Suppl. 77, 124.
Berfenstam, R. (1952). *Acta paediat., Stockh.* **41**, Suppl. 87, 3.
Bothwell, T. H., Pribilla, W. F., Mebust, W. & Finch, C. A. (1958). *Am. J. Physiol.* **193**, 615.
Brückmann, G. & Zondek, S. G. (1940). *Nature, Lond.* **146**, 30.
Cavell, P. A. & Widdowson, E. M. (1964). *Archs Dis. Childh.* **39**, 496.
Chang, L. L. (1973). *Acta paediat., Stockh.* **62**, 173.
Cunningham, I. J. (1931). *Biochem. J.* **25**, 1267.
Fay, J., Cartwright, G. E. & Wintrobe, M. M. (1949). *J. clin. Invest.* **28**, 487.
Fomon, S. J. (1967). *Infant Nutrition.* Philadelphia: W. B. Saunders.
Gamble, C. T., Hansard, S. L., Moss, B. R., Davis, D. J. & Lidvall, E. R. (1971). *J. Anim. Sci.* **32**, 84.
Krebs, H. A. (1928). *Klin. Wschr.* **7**, 584.
McFarlane, W. D. & Milne, H. I. (1934). *J. biol. Chem.* **107**, 309.
Markowitz, H., Gubler, C. J., Mahoney, J. P., Cartwright, G. E. & Wintrobe, M. M. (1955). *J. clin. Invest.* **34**, 1498.
Pommerenke, W. T., Hahn, P. F., Bale, W. F. & Balfour, W. M. (1942). *Am. J. Physiol.* **137**, 164.
Porter, H. (1966). In *The Biochemistry of Copper* p. 159 [J. Peisach, P. Aisen and W. E. Blumberg, editors]. New York: Academic Press.

Scheinberg, I. H., Cook, C. D. & Murphy, J. A. (1954). *J. clin. Invest.* **33**, 963.
Schulz, J. & Smith, N. J. (1958). *Am. J. Dis. Child.* **95**, 109.
Shaw, J. C. L. (1973). In *Nutritional Problems in a Changing World* p. 115 [D. Hollingsworth and M. Russell, editors]. London: Applied Science Publishers Ltd.
Shoden, A., Gabrio, B. W. & Finch, C. A. (1953). *J. biol. Chem.* **204**, 823.
Shrader, R. E. & Everson, G. J. (1967). *J. Nutr.* **91**, 453.
Spray, C. M. & Widdowson, E. M. (1950). *Br. J. Nutr.* **4**, 332.
Stevenson, S. S. (1943). *J. clin. Invest.* **22**, 403.
Tsai, H.-C. C. & Everson, G. J. (1967). *J. Nutr.* **91**, 447.
Vahlquist, B. C. (1941). *Acta paediat., Stockh.* **28**, Suppl. 5.
Vikbladh, I. (1951). *Scand. J. clin. Lab. Invest.* **3**, Suppl. 2.
Vosburgh, G. J. & Flexner, L. B. (1950). *Am. J. Physiol.* **161**, 202.
Widdowson, E. M., Chan, H., Harrison, G. E. & Milner, R. D. G. (1972). *Biol. Neonate* **20**, 360.
Widdowson, E. M. & Spray, C. M. (1951). *Archs Dis. Childh.* **26**, 205.
Wilkerson, V. A. (1934). *J. biol. Chem.* **104**, 541.

Printed in Great Britain