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copper neonate
diet zinc
maturation

Serum Copper and Zinc Concentration in Premature and Small-For-Date Infants

L. SANN,[41] D. RIGAL, G. GALY, F. BIENVENU, AND J. BOURGEOIS

Hopital Debrousse and Centre de Médecine Nucléaire, Lyon, France

Summary

Serum copper (Cu) and zinc (Zn) concentrations were measured in neonates with an appropriate birth weight for gestational age (AGA) and in small-for-gestational age infants. At 7 days of age, there was a positive correlation between serum Cu concentration and gestational age (GA) ($r = 0.63$; $P < 0.001$) and a negative correlation between Zn concentration and GA ($r = 0.62$; $P < 0.01$). At 7 days of age, the mean (± S.E.) concentrations in AGA full-term infants (Cu, 79 ± 8 μg/dl; Zn, 84 ± 4) were similar to those in small-for-gestational age, full-term infants (Cu, 78 ± 6 μg/dl; Zn, 85 ± 12). In preterm infants, there was also no difference between AGA and small-for-gestational age infants. In 23 AGA infants with a birth weight of less than 1500 g, serum Cu concentration increased from 51 ± 7 μg/dl at the age of 7 days to 86 ± 7 μg/dl at the age of 60 days (paired *t*-test: $P < 0.05$) whereas serum Zn concentration decreased from 149 ± 9 to 91 ± 5 μg/dl ($P < 0.01$). A positive correlation was found between serum Zn concentration and daily intake of Zn ($n = 39$; $r = 0.3458$; $P < 0.05$), but no correlation was found for serum Cu concentration. The evolution of serum Cu and Zn concentration with total age (GA + postnatal age) in the infants with a low birth weight (*i.e.*, <1500 g) was similar to the evolution with GA.

Speculation

In very-low-birth-weight infants, serum levels of Cu are influenced by the maturation whereas serum Zn concentrations depends also upon the dietary intake of Zn.

Zinc (Zn) and copper (Cu) are trace metals involved in many metabolic processes and enzyme systems (15) (36). Experimental investigations (29) (19) and studies in man (16) have shown that these metals are important for the development of fetus and particularly for its body growth and for the development of central nervous system and its myelination and for bone mineralization. The human fetus accumulates 70% of its Cu and Zn body stores during the last 12 wk of gestation (34). The infant born prematurely has small amounts of Cu and Zn and may not develop his body stores in a way similar to the fetus (7). However there are only a few reports of blood Cu and/or Zn concentrations in preterm infants (3, 23, 38, 39). There is no systematic investigation of their levels in normal preterm infants because most of the recent determinations have been carried out on preterm infants receiving parenteral nutrition (21, 26, 35). The purpose of the present study was to measure the serum concentration of Cu and Zn to determine the effect of gestational age and of dysmaturity on their levels just 7 days after birth. We also studied the postnatal changes of these two trace metals in preterm infants with a very low birth weight.

PATIENTS AND METHODS

PATIENTS

The 88 infants included in the present investigation were all admitted to Hopital Debrousse either because of their low birth weight or for systematic controls. None of them was seriously ill during the period of investigation. Their gestational age was assessed according to the last menstrual bleeding and when necessary by the score of Dubowitz *et al.* (9). An infant was considered as a small-for-gestational age (SGA) when his birth weight was below the tenth percentile of Lubchenco's growth chart (24). The main clinical data are given in Table 1. Of the twenty-one full-term neonates, 12 had an appropriate birth weight for their gestational age (AGA). Among the 67 preterm infants, 59 had a low birth weight (*i.e.*, 1.500 g or less). The postnatal changes of serum Cu and Zn concentration were measured in 31 of these low-birth-weight infants.

FEEDING OF THE INFANTS

Feeding was started in all infants during the first day of life with pooled pasteurized breast milk. Milk was given through polypropylene nasogastric tubes (Vygon) in all infants with a low birth weight and by bottle when the infants were able to suck. Vitamin A (12,000 IU/day), vitamin C (50 mg/day), and vitamin D_2 (2400 IU/day) were added to milk from the age of 10 days. A humanized formula was added to breast milk, usually at the end of the first month of life in AGA preterm infants or earlier in two AGA infants and in all SGA infants in order to provide adequate amounts for their appetite. The amounts of food are shown in Figure 1. No infant was supplemented with iron, and no sample was collected after blood transfusion.

HANDLING OF THE SAMPLES

Blood samples were drawn in the morning at the age of 7 days in all infants. In the 31 infants of the sequential study, the samples were collected at the age of 15, 30, 45, and 60 days. The infant's heel was pricked with a stainless steel lancet, and 500 μl of blood were collected in a polystyrene microtube. After clotting, the blood was centrifuged, and the serum was removed and placed into a plastic tube. The specimens were stored at +4°C until assayed. Hemolyzed samples were excluded. All analyses were performed on model 303 atomic absorption instruments (Perkin-Elmer Corp). After dilution by HNO_3 (10 mM), serum Cu concentration was measured with a HGA 74 cuvette by the method of Evenson and Warren (12). Serum Zn concentration was measured by flame spectrophotometry after dilution in 4% trichloroacetic acid and according to the method of Parker *et al.* (4, 28). The normal concentration (mean ± S.D.) in adults is 110 ± 20 μg/dl for Cu and 130 ± 30 μg/dl for Zn.

The concentration of Cu and Zn was also analysed in 29 samples of pooled pasteurized breast milk collected in a milk bank after the tenth day of lactation. It was drawn from a sterile glass feeding bottle or from polystyrene syringes. The weighed aliquots of breast milk were ashed in a muffle furnace at 500°C for 3 hr. Cu and Zn concentrations were measured on acid extracts of the ash by atomic absorption spectrophotometry.

SIGNIFICANCE OF THE RESULTS

The results will be expressed as mean ± S.E. Statistical analysis were performed using Student's *t* test, paired *t* test, and regression analysis.

Table 1. *Main clinical data*

	No.	Male	Female	Birth wt (g)	Gestational age (wk)	Twins	Toxemia
Full-term infants							
AGA	12	6	6	3250 (2940–3950)	40.8 (39–42)		
SGA	9	4	5	2120 (1630–2450)	39.1 (38–41)	5	1
Preterm infants							
Birth weight > 1500 g = AGA	8	4	4	1980 (1630–2630)	36.2 (35–37)		2
Birth weight ≤ 1500 g							
AGA	43	20	24	1247 (850–1500)	30.1 (27–34)		8
SGA	16	6	10	1245 (700–1400)	34.3 (31–37)	5	7
Sequential study							
AGA	23	12	11	1285 (850–1500)	30.5 (27–34)		2
SGA	8	5	3	1219 (1000–1400)	33 (32–35)	2	1

RESULTS

COMPOSITION OF MILK AND DAILY INTAKE

The concentration of pooled pasteurized breast milk in copper was 46 ± 3.3 μg/dl and in zinc was 454 ± 29.5 μg/dl. In the formula, the mean concentration given by the laboratory was 20 μg/dl for Cu and 120 μg/dl for Zn.

The total volume of milk and the intake of Cu and Zn ingested by the AGA and SGA infants with a low birth weight are shown in Fig. 1. In the AGA infants, breast milk represented 90% of total milk intake at 15 days of age and 45% at the age of 60 days. During the same time, mean Cu intake decreased by 17% ($P = 0.05$), and mean Zn intake decreased by 27% ($P < 0.025$). In SGA infants, breast milk represented 50% of total milk ingestion at the age of 15 days and 36% at the age of 60 days. At the same time, the decrease in Cu (5%) and Zn (13%) intakes were not significant.

Cu

Serum Concentration at the Age of 7 Days. The relationship to gestational age is shown in Figure 2. A positive correlation was found between serum Cu concentration and gestational age ($n = 53$; $r = 0.63$; $P < 0.01$). A comparison was made also between AGA and SGA infants. In SGA full-term infants, serum Cu concentration was 78 ± 6 μg/dl *versus* 79 ± 8 μg/dl in AGA full-term infants. In preterm infants when a comparison was made with the same gestational age or the same birth weight (Table 2), no difference could be detected between AGA and SGA infants.

Postnatal Evolution in Low-birth-Weight Infants. The postnatal evolution of serum Cu concentration in 23 AGA preterm infants is shown in Figure 3. It increased from 51 ± 7 μg/dl at the age of 7 days to 86 ± 7 μg/dl at the age of 60 days (paired t test; $P < 0.05$). In 8 SGA infants, the increase from 62 ± 13 to 73 ± 20 μg/dl was not significant. In AGA and SGA infants, there was no correlation between the daily intake and the serum level of Cu.

Comparison between Postnatal and Gestational Evolution. Figure 4 shows that the gestational and postnatal evolutions of serum Cu concentration were similar when the postnatal evolution is expressed in total age (gestational age plus postnatal age).

Zn

Serum Concentration at the Age of 7 Days. The relationship to gestational age is shown in Figure 2. A negative correlation was observed between serum Zn concentration and gestational age ($n = 39$; $r = 0.62$; $P < 0.01$). When a comparison was made between AGA and SGA infants, no difference was found in full-term infants: 84 ± 4 in 12 AGA infants *versus* 81 ± 12 μg/dl in 9 SGA infants. In preterm infants, no difference was detected either in infants with the same gestational age or with the same birth weight (Table 2).

Postnatal Evolution in Low-Birth-Weight Infants. In 23 AGA preterm infants, serum Zn concentration decreased between the age of 7 days and the age of 60 days from 149 ± 9 to 91 ± 5 μg/dl (paired t test; $P < 0.01$). In 8 SGA infants, a similar decrease from 132 ± 28 to 70 ± 3 μg/dl was observed (paired t test; $P < 0.01$). There was a positive correlation between these concentrations and the dietary intake of Zn in the AGA infants: serum Zn = 0.084 × daily intake + 56 ($n = 39$; $r = 0.3458$; $P < 0.05$) and a better correlation in the SGA infants: serum Zn = 0.094 × daily intake + 39 ($n = 10$; $r = 0.6776$; $P < 0.05$).

Comparison Between Postnatal and Gestational Evolution. As shown in Figure 4, the curves of the means were almost similar for the gestational age and for the postnatal age expressed in total age (*i.e.*, gestational age plus postnatal age).

DISCUSSION

The interpretation of serum Cu and Zn levels depends on many factors: firstly, metabolic factors such as dietary intake, intestinal absorption, digestive and urinary excretion; secondly, plasmatic and tissue distribution; thirdly, neonatal maturation. In the present investigation, only three of these factors could be studied: maturation, dysmaturity, and the role of dietary intake in low-birth-weight infants. These factors will be discussed separately for Cu and Zn.

Cu

In full-term infants, serum Cu concentrations rise steeply after birth (11, 17). The values observed at the age of 7 days in the present study were similar to those reported by Henkin *et al.* (18) but higher than those published by Ohtake (27) for term infants aged 5 days. These discrepancies may be explained by methodologic reasons. In preterm infants, previous studies of the evolution of serum Cu concentration with gestational age have displayed contradictory results. Lesné *et al.* (23) and Widdowson *et al.* (38) observed a decrease in human fetuses. In cord blood, Schenker *et al.* (33) observed no significant change between 32 and 36 wk of gestation and full-term infants. However, Canzler *et al.* (5) found an increase in the mean concentration in cord blood with gesta-

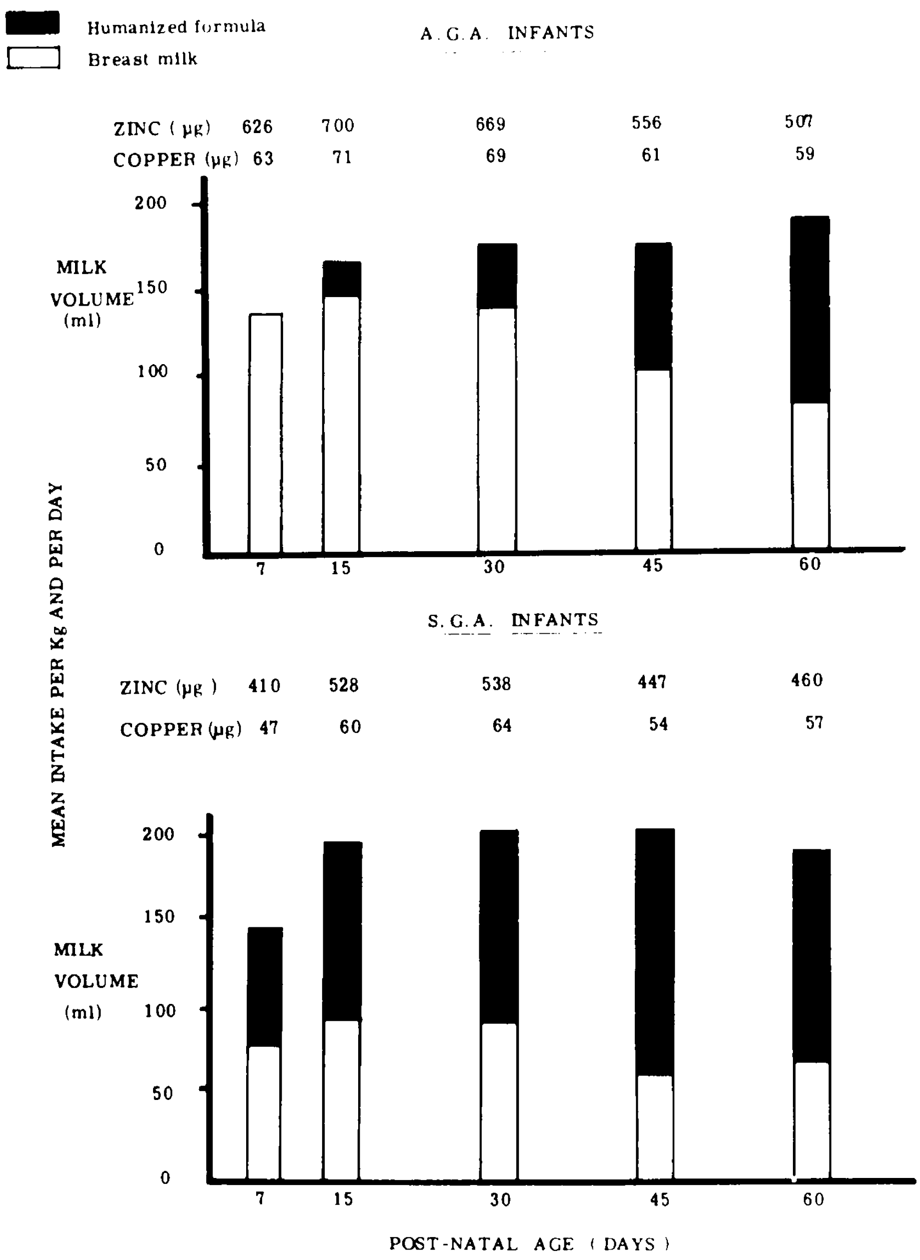


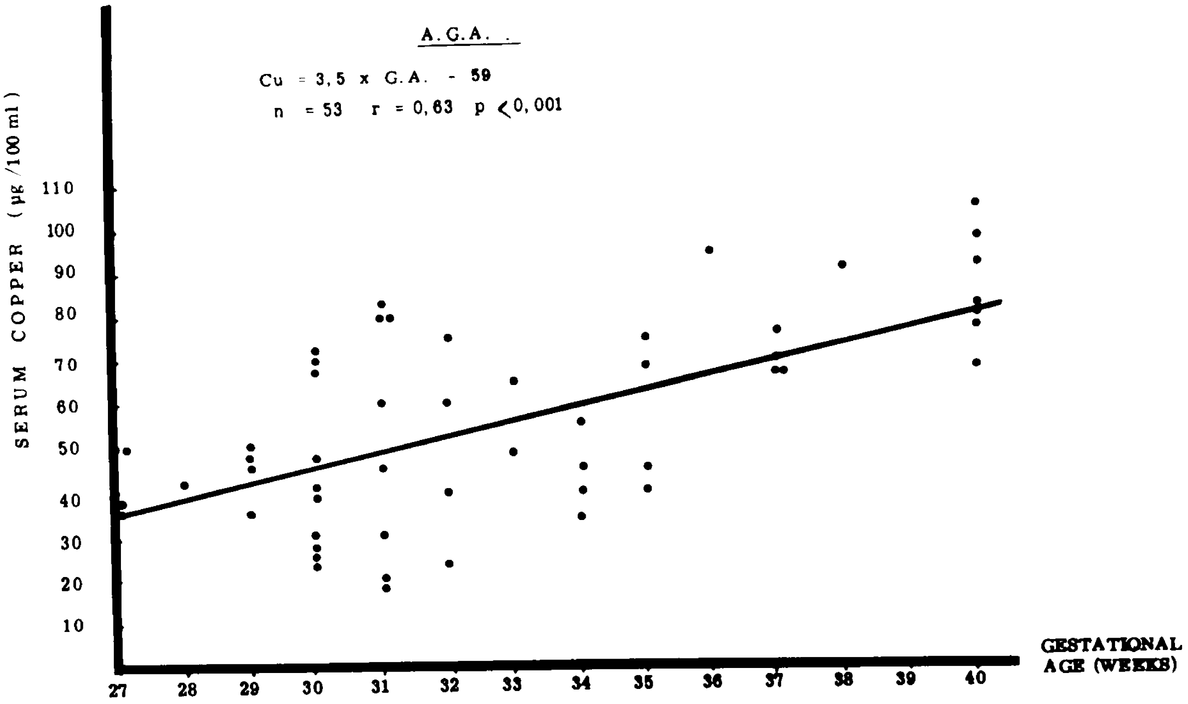
Fig. 1. Daily milk volume and Cu and Zn intake in the low-birth-weight infants.

tional age and a positive correlation with gestational age. The differences between these reports may be attributed to different methodologic procedures of patient selection and serum Cu determinations. In the present study, we found a positive correlation between gestational age and serum Cu concentration. Although this result is similar to the finding of Canzler *et al.* (5), it is, however, difficult to compare it with those of the literature because our determinations could be made only at the age of 7 days for practical reasons. Moreover, the evolution of serum Cu concentration during the 7 days after birth is not known in premature infants.

The role played by ceruleoplasmin in the positive correlation between gestational age and serum Cu concentration is also unknown. In fetuses, Gitlin and Biasucci (13) did not find any significant rise during the last trimester after its rapid increase at 27 wk of gestation. However, Canzler *et al.* (5) detected a positive correlation between serum Cu and ceruleoplasmin concentrations in cord blood of preterm infants. Preliminary determinations in our laboratory of plasma ceruleoplasmin concentration at the age of 7 days showed higher values in full-term infants (range, 20 to 29 mg/dl) than in preterm infants (10 to 15 mg/dl). Finally, the differences in Cu and ceruleoplasmin concentrations reported here between term and low-birth-weight infants at 7 days could be due to an absent or slower rise following birth in the low-birth weight infants.

The present results also show that at the age of 7 days the serum levels of Cu are similar in AGA and SGA infants. They suggest that dysmaturity has no influence on serum Cu levels. However a reduction of the body stores of copper cannot be excluded (1, 2, 32).

The postnatal increase in serum Cu concentration in the low birth weight infant could be explained by a retention of Cu because Dauncey *et al.* (7) observed a positive balance after the age of 35 days in preterm infants. However, the plasma Cu concentration increases in full-term infants after birth (17, 18) despite a low absorption of Cu (38). In the present study, the total Cu intake decreased with postnatal age, and the mean Cu intake throughout the study (65 μg/kg/24 hr) was less than the mean



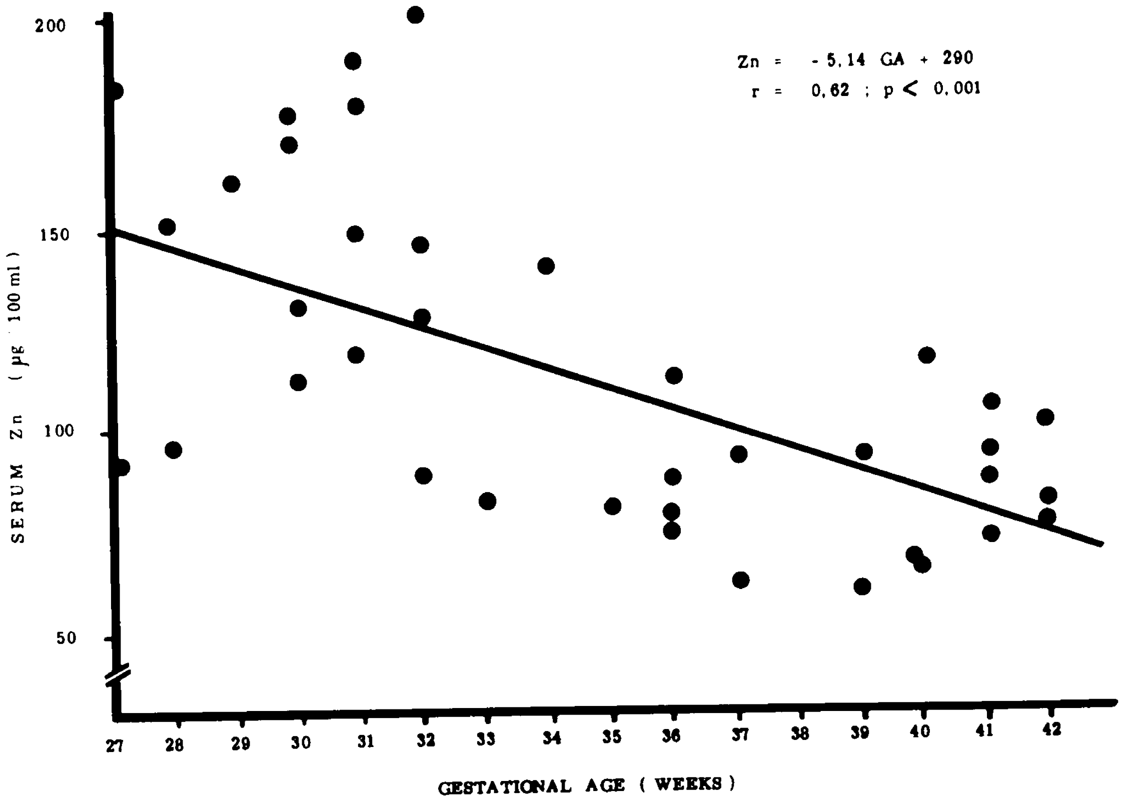


Fig. 2. Evolution of serum Cu and Zn concentrations with gestational age in AGA newborn infants.

Table 2. *Comparison of serum Cu and Zn concentration at the age of 7 days in AGA and SGA infants*

Condition (µ/dl)	Same gestational age (mean = 32.8; 31–34 wk)		Same birth wt (mean = 1200 g; 1000–1500 g)	
	AGA	SGA	AGA	SGA
Cu	68 ± 11[1] (14)[2]	62 ± 13 (9)	51 ± 5 (8)	78 ± 10 (10)
Zn	141 ± 10 (11)	132 ± 28 (6)	130 ± 15 (6)	136 ± 20 (7)

[1] Mean ± S.E.

[2] Numbers in parentheses, number of infants.

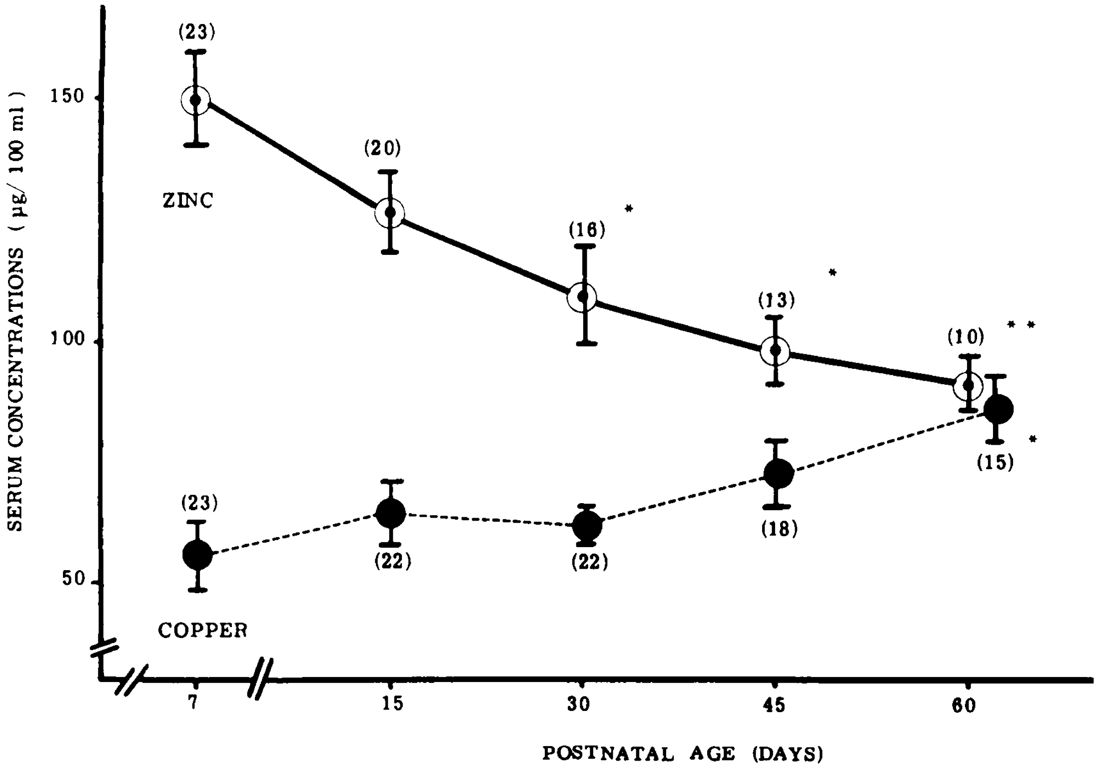


Fig. 3. Postnatal evolution of mean ± S.E. serum Cu and Zn concentrations in low-birth-weight AGA infants.

rate of Cu accumulation by the human fetus between 26 and 36 wk, 86 µg/kg/24 hr according to Shaw (34). In addition, there was no correlation between the dietary intake and serum Cu concentrations. These results agree with the findings of Wilson and Lahey (39) who could not induce hypocupremia in premature infants fed with low Cu intakes.

Finally, the present results suggest that in low-birth-weight infants, the serum concentration of Cu is related to maturation rather than to metabolism. In that respect, the role played by the maturation of ceruleoplasmin synthesis deserves further investigation.

Zn

In full-term infants, plasma Zn concentration shows little change during the first wk (3, 18, 27). The levels found in the present study are similar to those reported by Henkin *et al.* (17, 18) and Kurtz *et al.* (22).

The negative correlation between serum Zn concentrations and gestational age agrees with the scant results obtained in cord blood of human fetuses by Berfenstam (3) and Widdowson *et al.* (38). In serum, Zn is bound to serum albumin, transferrin, α2-macroglobulin, and amino acids, especially histidine, threonine, glutamine, cystine, and lysine (14). The decrease in serum Zn concentration cannot be attributed to the gestational evolution of serum albumin, transferrin, or α2-macroglobulin because their levels in cord blood of human fetuses increase with gestational age (13, 20). The relationship between serum Zn and plasma amino acids concentrations in fetus is difficult to assess because there are only a few reports of blood amino acid measurements in human fetuses (6, 31) and because the amount of Zn bound to amino acids in fetuses and newborn infants is not known.

In SGA infants, serum Zn concentrations were similar to the levels in AGA infants. These findings agree with unpublished results by Prasad (30) in full-term infant. Therefore, dysmaturity does not influence the serum concentration of Zn near birth.

The postnatal decrease in serum Zn concentration has never been shown in preterm infants fed PO. Previous studies were carried out only in infants placed on parenteral nutrition (21, 26, 35). This postnatal decrease could be attributed to three factors: gestational maturation, dietary intake, and metabolism. The present study shows that the postnatal evolution of serum Zn concentration is similar to the evolution with maturation. However, in full-term infants, Henkin *et al.* (18) also found a decrease in plasma Zn concentration during the first 3 months of life. These changes were related mainly to changes in nondiffusable macromolecular-linganded Zn. As discussed previously, the mechanism of this decrease is not known in premature infants. The present study shows that at least one part of this decrease can be attributed to the decrease in dietary Zn intake. This is an agreement with experimental studies in rats (8) and studies in early childhood (37) showing that a Zn-deficient diet can produce a rapid fall in plasma

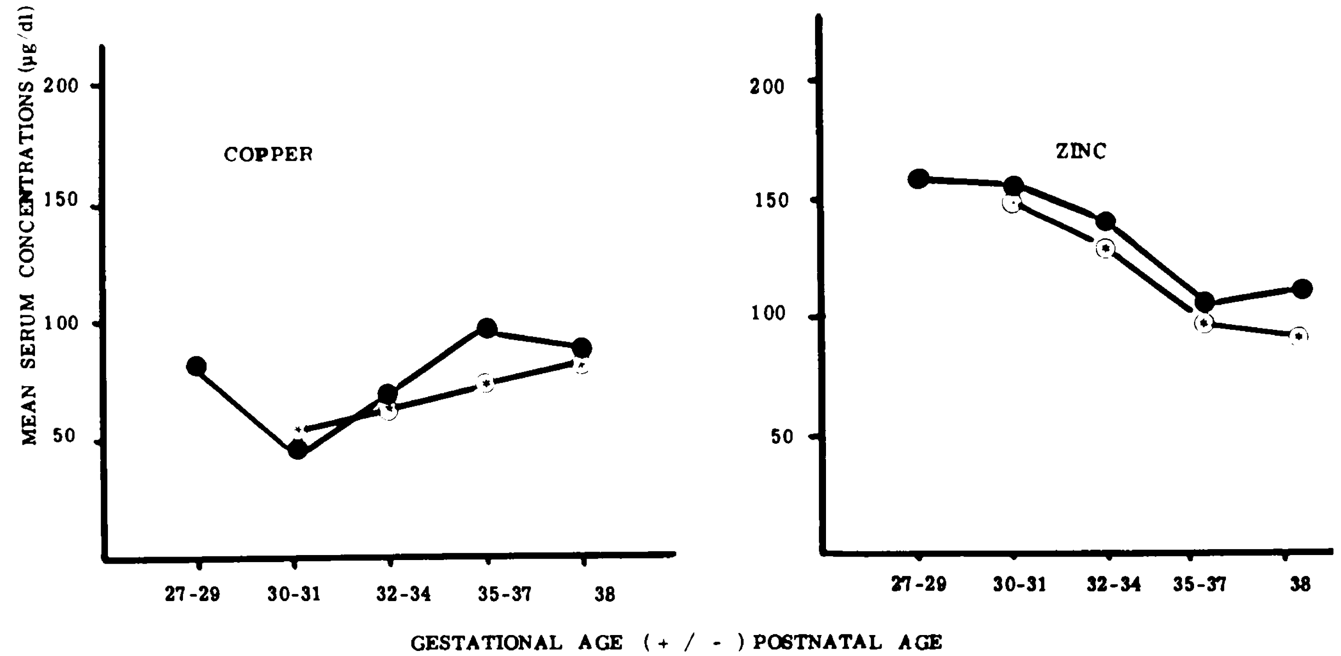


Fig. 4. Comparison between evolution of serum Cu and Zn concentration with gestational age (●) and with total age (gestational age plus postnatal age) (⊙) in preterm infants.

Zn concentration. The postnatal decrease in serum Zn concentration also agrees with metabolic studies because Dauncey *et al.* (7) found a negative balance of Zn during the first 60 days of life in low birth weight infants. This negative balance was attributed to a failure to reabsorb their endogenous Zn because parenterally administrated Zn may appear in stools rather than in urine (25). A Zn-binding ligand is present in breast milk but not in cow's milk (10). Its concentration in pasteurized breast milk could play an important role on Zn absorption in premature infants.

Finally, the present results suggest that in contrast to serum Cu, the postnatal evolution of serum Zn concentration is in agreement with metabolic studies in preterm infants.

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40. Informed consent was obtained from the parents of the patients studied.
41. Requests for reprints should be addressed to: Dr. L. Sann, Hopital Debrousse, Centre de Medicine Nucleaire, Lyon, France.

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