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Polyamine Concentration in Rat Milk and Food, Human Milk, and Infant Formulas[1]

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ABSTRACT. The polyamine concentration in rat milk and food, human milk, and infant formulas was estimated by HPLC. In rat milk, the concentration of putrescine and spermine was low (generally under 2.5 nmol·mL^{-1} for putrescine and under 1 nmol·mL^{-1} for spermine). The spermidine concentration was higher and seemed to increase during lactation. The rat food was richer in polyamines than the rat milk (about 150 times for putrescine and spermine, about 30 times for spermidine). We already proved that ingestion of spermine or spermidine can induce precocious maturation of the rat intestine. The present observations suggest that polyamines contained in rat food could play an important role in postnatal maturation of the rat intestine. The polyamine concentration of human milk was measured from 60 different mothers during a period extending from the 1st wk to the 6th mo of lactation. Great variation was observed. During the 1st mo of lactation, the general pattern was as follows: putrescine concentration generally varied little (from 1 to 3 nmol·mL^{-1}), spermine and spermidine concentrations showed a similar pattern (the highest values appeared at the end of the 1st wk of suckling). After the 4th mo of lactation, putrescine concentration increased slightly, whereas spermine and spermidine concentration stayed almost stable. The concentrations of polyamines in 18 powdered milks for babies were estimated. Spermine and spermidine contents were lower than those in human milk. A protective effect of spermine or spermidine against alimentary allergies is suggested. (*Pediatr Res* 32: 58–63, 1992)

In the rat, maturation of the gastrointestinal tract occurs during the 3rd postnatal wk (for example, see 1–4). Before maturation, the lactase sp act of the small bowel mucosa is high, whereas sucrase and maltase are very low. This immature mucosa is characterized histologically by the presence of enterocytes containing a large supranuclear vacuole and an apical canalicular system probably involved in nonspecific protein transfer through the gut wall. At the moment of maturation (18th-20th postnatal d, *i.e.* the time of weaning) mucosal sp act of maltase and sucrase increase dramatically, whereas lactase sp act decreases to very low values. At the same time, enterocytes lose their apical canalicular system as well as their supranuclear vacuole and nonspecific protein absorption ceases ("gut closure"). Various attempts have been made to influence this development sequence (5–12). Whereas various hormones or local mediators have been shown to be involved in the regulatory mechanism of intestinal maturation in suckling rodents, the influence of food or food constitutents has been less thoroughly studied.

In previous studies (13–15), we showed that it was possible to induce precocious intestinal maturation in the rat by giving spermidine or spermine orally, *i.e.* polycationic substances of low molecular weight that are ubiquitous and are synthesized by all cell types studied. These polyamines were chosen because it is known that these molecules are involved in intestinal growth (16). They are synthesized locally through the induction of ornithine decarboxylase, which is the first enzyme involved in polyamine synthesis. Another reason for our choice was that it has been proved (8) that mucosal polyamine concentrations show a dramatic increase at the moment of postnatal intestinal maturation in the rat. To precisely state the meaning of our observations, we decided to analyze the polyamine content of the rat milk and food. These results are reported below.

Because it is important to know the significance of our findings for human health, we measured the polyamine concentration in infant formulas and in human milk. A high polyamine content in the food of babies could, indeed, be very important for favoring the final maturation steps of their intestine: it is now generally accepted that the intestine of healthy term infants is more permeable to food proteins during the first 3 mo than later in life (17–19); it is acknowledged that, during the period that follows birth, antigenic macromolecules can penetrate the small intestinal mucosal membranes in quantities that may be of immunologic importance (18, 20, 21).

MATERIALS AND METHODS

Polyamine concentrations. The polyamine concentrations in the various solutions analyzed were measured by HPLC (22). Solid sulfosalicylic acid (20 mg) was added to 300 μL of milk and 300 μL of distilled water (MilliQ; Waters Assoc., Millipore Corp., Bedford, MA) or to 50 mg of rat food suspended in 1 mL of water. Protein precipitation was obtained by leaving the samples overnight at −20°C. After centrifugation at 3000 × *g* for 15 min, aliquots of 200 μL of supernatants were used for derivatization.

Dansylation was carried out as described by Bontemps *et al.* (22). The reproducibility of extraction was tested. It was greater than 95% for spermine and spermidine and greater than 75% for putrescine. Repeated injections showed a coefficient of variation lower than 1.5%. The dansylated derivatives of polyamines were separated on a reversed-phase column (Lichrocart RP-18; Merck, Darmstadt, Germany). The polyamine concentrations were expressed in nmol·mL^{-1} and the polyamine contents in nmol·g^{-1}.

Rat milk. Wistar rats were housed in standard conditions. They had free access to water and food (R/S A03, UAR, Epinay-

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sur-Orge, France). The young rats were reared by their natural mothers in the absence of other animals. The room was kept at 23°C with 12 h of daylight.

The polyamine contents of the milk of nine female rats were determined. Milk samples were taken, every 3 d, in the morning throughout the whole lactating period. The she-rat was separated from her young for the night (± 16 h). Then, she was anesthetized. To numb the rat, anesthetic (Ketalar, Warner-Lambert, Zaventem, Belgium) was intraperitoneally injected (0.1 mL·100 g^{-1} body weight).

To stimulate milk secretion, oxytocin was subsequently injected intraperitoneally [Syntocinon (Sandoz, Basel, Switzerland): 1 U·mL^{-1}; 0.1 mL·100 g^{-1} body weight]. Milk was extracted by a homemade breast pump; 0.5 to 2 mL of milk were collected.

These experiments were performed with the highest standards of humane care with the approval by local committee on animal investigation.

Rat food compounds. Complete rat food (R/S A03) was crushed into powder using a pestle and mortar. Then, 50 mg of rat food powder was suspended in 1 mL of water containing 10 mg of sulfosalicylic acid. This concentration was chosen after several trials. Such a preparation was made twice. Aliquots were kept at −20°C for at least one night. Dansylation of both extracts was carried out as described above. Two measurements (double injection on HPLC system) were made for each extract.

Human milk. Polyamine concentrations of human milk were determined during the first 6 postnatal mo. When possible, a milk sample was collected each day during the 1st postnatal mo. Afterwards, it was obtained once a week until the end of lactation.

Preliminary studies were undertaken to find out the best moment of sampling during one suckling. Then, the milk samples were collected in a standardized way with the help of the staff of the milk bank of Liege and individual mothers. Milk aliquots were frozen immediately after gathering and kept at −20°C until analysis.

Infant formulas. We measured polyamine concentrations in most commercial infant formulas available on the Belgian market. These powders were diluted as indicated by the manufacturer, just before polyamine dansylation (see above). Two extracts were prepared and two measurements (double injection on HPLC system) were made for each extract.

Statistics. Two different methods were used to study the evolution of polyamine concentrations in rat milk during the entire lactating period (± 25 d). First, a separate regression line was fitted for the data obtained for each rat in which at least three consecutive measurements were available. Slopes and intercepts were then averaged and tested for significance by using *t* test. Secondly, a regression line was also fitted to all the time-related data, whether from the same or other rats, to assess the overall trend of the variables studied. A similar approach was used for data collected from women's milk. All results were considered to be significant at the 5% critical level.

RESULTS

Rat milk. There was no success in milking the she-rats the day after birth. Thus, results were obtained for the period between the 2nd and the 25th d after birth. Transversal (or overall) and longitudinal (or individual) analyses of data are reported in Table 1. The measurements showed great individual variability for each polyamine contained in rat milk (transversal analysis). The concentration of putrescine (a diamine) and spermine was quite low (under 2.5 nmol·mL^{-1}), except for putrescine in milk secreted at the beginning of lactation, and did not vary much during the lactating period. Longitudinal analyses confirm these observations. The mean of spermidine concentration was higher and seemed to increase during lactation (± 9 to >20 nmol·mL^{-1}), but, looking at individual serial data, we were not able to confirm this finding, possibly because of the limited amount of data.

Rat food. As shown in Table 1, the complete rat food contained large quantities of polyamines.

Human milk. To choose the best conditions to take milk samples, we carried out preliminary studies: *1*) estimation of the polyamine concentrations in milk throughout one suckling; *2*) evolution of putrescine, spermidine, and spermine concentrations in the course of 1 d of the 3rd wk of lactation; *3*) analysis of polyamine concentration in milk samples taken from both breasts at the end of suckling. These studies showed a great variation in concentrations of spermine and spermidine from one mother to another and differences in the concentration of polyamines between the left and the right breast of a particular mother. Because it appeared that it is psychologically inadvisable to interrupt a baby during breast suckling and that the results may be overvalued by taking a sample at the end of suckling, we decided to take milk from the 2nd breast before the baby began suckling it (under these conditions, contraction of the breast had already occurred).

Tables 2 and 3 show the results obtained for 60 different mothers. Table 2 concerns the everyday averages for the 1st mo of lactation and the longitudinal analysis performed in two groups of women (2–12 d after birth and 12–31 d after birth). Table 3 shows the averages of the monthly measurements (obtained once a week) when analyzing milks secreted from the 2nd mo to the 6th mo of lactation (transversal analysis) and the results of the longitudinal analysis performed with the data obtained. Great individual variation was observed.

The general pattern of polyamine concentrations in human milk during the 1st mo of lactation obtained from the longitudinal analysis (Table 2) indicated that: *1*) putrescine concentration did not significantly vary, and *2*) spermidine and spermine concentrations showed a similar pattern. The highest values appear at the end of the 1st wk of suckling. Some mothers always seem to have quite high or quite low concentrations of putrescine, spermine, and spermidine, which show the same tendency to decrease at the end of the 1st mo of lactation. However, a firm conclusion could not be drawn. The statistical analysis did not reveal a significant regression slope.

Individual regressions fitted to data obtained between the 5th wk and the 20th wk of lactation (Table 3) showed that putrescine concentration increased, whereas spermine and spermidine concentration stayed almost constant in human milk. Spermine and spermidine concentration had a tendency to decrease, but not significantly.

Infant formulas. We measured the concentration of polyamines in 18 powdered milks for babies (Table 4). The analyses showed that the concentrations of spermine, spermidine, and putrescine in all tested samples of milk varied between 0 and 0.87, 0 and 2.78, and 0.62 and 6.07 nmol·mL^{-1}, respectively.

DISCUSSION

In a previous publication (14), we reported that it is possible to induce precocious intestinal maturation in rats by giving them spermine or spermidine orally. This observation was recently confirmed by another research team (23).

To find out if ingested polyamines can play a role in the normal development of the rat intestine, we estimated the concentration of these substances in rat milk and food.

Our results (Table 1) show that in rat milk the concentration of putrescine and spermine was quite low (less than 2.5 nmol·mL^{-1}) (transversal analysis), as pointed out by Pollack *et al.* (24). This concentration did not vary much during the lactating period (longitudinal analysis), when spermidine concentration was higher and could increase during lactation (from 9 to >20 nmol·mL^{-1}).

As shown by the data reported in Table 1, rat food was richer in polyamines than rat milk (approximately 150 times for pu-

Table 1. *Polyamine concentrations (nmol·mL^{-1}) in rat milk during the whole lactating period**

1. Transversal analysis

Day after birth	Putrescine [$x \pm$ SEM (n)]	Spermidine [$x \pm$ SEM (n)]	Spermine [$x \pm$ SEM (n)]
1–3	4.25 ± 1.94 (5)	11.11 ± 1.72 (5)	0.38 ± 0.03 (5)
4–6	1.58 ± 0.48 (6)	8.94 ± 0.78 (6)	0.27 ± 0.07 (6)
7–9	2.21 ± 0.63 (6)	13.10 ± 3.72 (7)	0.53 ± 0.10 (7)
10–12	0.87 ± 0.59 (6)	11.91 ± 2.76 (8)	0.57 ± 0.18 (8)
13–15	1.00 ± 0.33 (8)	15.12 ± 2.30 (10)	0.60 ± 0.08 (10)
16–18	1.60 ± 0.62 (6)	23.70 ± 4.74 (9)	0.89 ± 0.17 (10)
19–21	0.83 ± 0.28 (6)	20.50 ± 2.72 (9)	0.91 ± 0.08 (8)
22–25	1.55 ± 0.54 (5)	21.47 ± 8.51 (5)	1.12 ± 0.40 (5)
Rat food	223	442	104
	209	428	100

2. Longitudinal analysis: slopes (b) and intercepts (a) expressed as mean ± SEM

Putrescine: a = 0.602 ± 0.465	Spermidine: a = 11.195 ± 5.761	Spermine: a = 0.407 ± 0.182
b = −0.013 ± 0.035	b = 0.273 ± 0.304	b = 0.018 ± 0.009

Hypothesis that slope is zero cannot be rejected at the 5% critical level (other explanations in the text)

* n = number of milkings. Polyamine content in rat food is given in nmol·g^{-1}.

trescine and for spermine, and approximately 30 times for spermidine). Although it may be argued that the wide variation in values obtained when measuring polyamine concentration in rat milk (Table 1) could be due to the treatment of the she-rats to obtain milk and although it is well known that rat feeding does not change abruptly at weaning, it may be suggested that the polyamines found in solid rat food represent a significant increase in the exogenous contribution of these substances in the rat at this moment. The polyamines contained in the solid rat food could thus play an important role in the postnatal maturation of the rat intestine or in the maintenance of a functional stage of the rodent bowel. The latter possibility agrees with results obtained by other authors (16). This hypothesis is further reinforced by the fact that the bacterial flora, another source of exogenous polyamines, grows with the administration of solid food (25).

To evaluate the possible implications of our findings for humans, we measured the polyamine concentration in infant formulas and in human milk.

The following considerations prompted us to carry out these analyses: *1*) it is well known that babies often have a high intestinal permeability for food proteins (17, 19, 26), which could lead to the development of allergic reactions (18), and that rat intestinal maturation is characterized by a decrease in intestinal permeability for macromolecules (4, 27); *2*) it has been noted that allergy problems seem to be less frequent in breast-fed children when compared with bottle-fed infants (for example, see 18). This observation suggests that when compared with human milk, infant formulas might lack one or several compounds important for intestinal maturation, for example spermine or spermidine.

Preliminary results showed that great variation exists in the concentration of milk polyamines from one mother to another. Even for a given mother, such variation was observed according to the time of day, the stage of suckling, or the breast chosen. It has also been observed for other milk components (for a review, see 28 and 29).

To avoid psychologic problems for the mother and her baby and to reduce the apparent variation in the milk-polyamine concentrations, we decided to perform a systematic analysis by taking milk samples from the second breast just before the baby began sucking it.

Great individual variation was recorded (Tables 2 and 3). We can nevertheless determine the general pattern of polyamine concentrations during lactation from individual regression analyses. Polyamine concentration varied little throughout the lactating period. Spermine and spermidine concentrations seemed to have higher values at the end of the 1st wk of suckling than at the end of the 1st mo of lactation. A similar observation was partly reported elsewhere by other authors (30). After the 5th wk of lactation, putrescine concentration increased slightly in milk, whereas spermine and spermidine contents were almost constant but had a tendency to decrease. Several mothers seem to have consistently quite high or quite low concentrations of putrescine, spermine, and spermidine. These individual variations may be due, for example, to the diet, the way of life, or the genetic background of each mother. If our reasoning concerning the role of polyamines in the prevention of allergies is correct, a parallel could be established between low concentrations of polyamines in breast milk and a predisposition of children to become allergic. To check this hypothesis, a long-term follow-up of these infants will be undertaken.

The polyamine concentration in 18 powdered milks for babies was measured (Table 4). The putrescine concentration was highly variable but had values that were sometimes higher than those of human milk (Tables 2 and 3). The spermine concentration was always lower in infant formula than in human milk. The spermidine concentration of human milk was also higher than that of infant formula (Tables 2–4), as pointed out by other authors (12).

The results presented above clearly indicate that the spermine and spermidine intake of breast-fed babies is higher than that of infant formula-fed babies. If our observations showing that spermine or spermidine ingestion induces precocious intestinal maturation of the intestine in rat (14) could be extended to man, they could mean that breast-feeding favors intestinal maturation in babies, for example, by bringing about a decrease in protein permeability.

It may, of course, be argued that polyamine metabolism in the intestinal mucosa enables natural maturation to occur, especially because the putrescine concentration of powdered milk is not very different from that of mother's milk. Such a possibility may not be ruled out. However, we have to remember that we showed that putrescine ingestion in unweaned mice did not induce intestinal maturation (31). On the contrary, we noticed signs of partial involution of the intestinal epithelium.

From our results, it may be proposed that the possible protective effect of human milk against allergies, which has been reported in the literature (for example, see 18), could be explained at least partially by its high level of polyamines, especially spermine (see above).

Such a hypothesis is reinforced by other observations that we made and summarized elsewhere (13) indicating that pancreatic

Table 2. *Polyamine concentrations (nmol·mL^{-1}) in human milk during the 1st mo of lactation**

1. Transversal analysis

Day after birth	Putrescine [$x \pm$ SEM (n)]	Spermidine [$x \pm$ SEM (n)]	Spermine [$x \pm$ SEM (n)]
2	0.72 (1)	3.73 (1)	3.12 (1)
3	2.04 ± 0.86 (9)	3.58 ± 0.73 (9)	3.25 ± 0.48 (9)
4	1.87 ± 0.53 (13)	2.99 ± 0.44 (13)	3.45 ± 0.61 (13)
5	1.80 ± 0.94 (13)	4.14 ± 0.76 (13)	4.44 ± 1.06 (13)
6	1.03 ± 0.13 (13)	5.06 ± 0.64 (13)	5.22 ± 0.71 (13)
7	1.29 ± 0.21 (12)	7.11 ± 1.09 (12)	6.63 ± 1.36 (12)
8	1.36 ± 0.19 (12)	9.11 ± 1.46 (12)	6.89 ± 1.15 (13)
9	1.75 ± 0.40 (13)	8.75 ± 1.47 (13)	7.38 ± 1.36 (14)
10	1.02 ± 0.17 (16)	7.44 ± 0.91 (16)	8.29 ± 1.32 (16)
11	1.34 ± 0.23 (14)	8.00 ± 1.29 (15)	7.71 ± 1.25 (15)
12	1.10 ± 0.11 (19)	6.91 ± 0.70 (21)	6.77 ± 0.95 (20)
13	1.26 ± 0.19 (19)	8.17 ± 1.09 (19)	7.75 ± 1.23 (19)
14	1.08 ± 0.14 (19)	5.69 ± 1.12 (19)	6.13 ± 1.54 (18)
15	1.59 ± 0.28 (16)	7.31 ± 1.22 (17)	8.23 ± 1.65 (16)
16	1.00 ± 0.13 (14)	5.40 ± 0.87 (16)	6.13 ± 1.09 (14)
17	1.09 ± 0.14 (17)	6.89 ± 1.00 (17)	7.49 ± 1.32 (17)
18	1.12 ± 0.08 (16)	6.71 ± 1.19 (16)	6.30 ± 1.03 (15)
19	1.25 ± 1.17 (15)	7.41 ± 1.83 (15)	8.07 ± 2.20 (16)
20	1.27 ± 0.22 (15)	5.17 ± 0.59 (16)	6.06 ± 1.57 (15)
21	1.07 ± 0.23 (13)	6.14 ± 1.19 (12)	6.35 ± 1.34 (13)
22	1.13 ± 0.18 (13)	5.78 ± 1.13 (13)	5.40 ± 1.39 (12)
23	1.43 ± 0.75 (13)	6.64 ± 1.10 (12)	8.06 ± 1.54 (11)
24	1.79 ± 0.49 (12)	6.98 ± 1.52 (11)	7.60 ± 1.48 (10)
25	2.15 ± 0.85 (12)	6.07 ± 1.07 (12)	5.67 ± 1.03 (10)
26	2.39 ± 0.81 (11)	4.02 ± 0.94 (10)	5.18 ± 1.08 (8)
27	1.81 ± 0.66 (9)	4.30 ± 0.97 (8)	5.28 ± 0.83 (6)
28	1.20 ± 0.22 (9)	2.94 ± 0.58 (8)	4.19 ± 1.05 (7)
29	1.56 ± 0.49 (8)	5.29 ± 0.85 (6)	4.37 ± 0.75 (7)
30	1.35 ± 0.27 (6)	3.96 ± 0.18 (4)	4.67 ± 0.51 (5)
31	1.34 ± 0.22 (5)	6.75 ± 0.14 (3)	5.83 ± 0.82 (4)

2. Longitudinal analysis in two groups of women (2–12) and (12–31): slopes (b) and intercepts (a) are expressed as mean ± SEM

Putrescine (2–12):	a = 1.295 ± 0.895	Putrescine (12–31):	a = 0.880 ± 0.551
	b = −0.019 ± 0.083		b = 0.026 ± 0.033
Spermidine (2–12):	a = 3.021 ± 1.423	Spermidine (12–31):	a = 9.624 ± 2.535
	b = 0.416 ± 0.197		b = −0.205 ± 0.119
Spermine (2–12):	a = 3.868 ± 1.907	Spermine (12–31):	a = 8.366 ± 2.929
	b = 0.309 ± 0.239		b = −0.129 ± 0.137

Hypothesis that slope is zero cannot be rejected at the 5% critical level (other explanations in the text)

* n = number of samples (one sample for one mother).

Table 3. *Polyamine concentrations (nmol·mL^{-1}) of human milk from the 2nd to the 6th mo of lactation**

1. Transversal analysis

Months after birth	Putrescine [$x \pm$ SEM (n)]	Spermidine [$x \pm$ SEM (n)]	Spermine [$x \pm$ SEM (n)]
2	0.90 ± 0.12 (13)	3.85 ± 0.85 (13)	3.44 ± 0.72 (13)
3	1.10 ± 0.14 (5)	2.08 ± 0.50 (5)	2.78 ± 1.85 (5)
4	0.79 ± 0.15 (7)	3.16 ± 0.54 (7)	3.50 ± 1.01 (7)
5	0.84 ± 0.16 (2)	1.85 ± 0.70 (2)	2.15 ± 1.46 (2)
6	0.87 ± 0.35 (5)	2.98 ± 1.40 (5)	2.33 ± 1.15 (5)

2. Longitudinal analysis: slopes (b) and intercepts (a) expressed as mean ± SEM

Putrescine:	a = −1.330 ± 0.574	Spermidine:	a = 4.947 ± 2.418	Spermine:	a = 1.938 ± 2.408
	b = 0.183 ± 0.066		b = −0.217 ± 0.213		b = −0.195 ± 0.459

Slope significantly increases in the case of putrescine

Hypothesis that slope is zero cannot be rejected at the 5% critical level in the case of spermidine and spermine

* x = mean of the results obtained during the indicated month; n = number of samples.

Table 4. *Polyamine concentrations in infant formulas**

	Putrescine	Spermidine	Spermine
Nutricia			
Peptijunior	1.07	0.07	0.08
	0.86	0.11	0.11
Nutrisoja	0.84	2.33	0.44
	0.64	2.78	0.43
Nutrimel	3.32	0.24	0.13
	2.61	0.15	0.18
Almiron 1	1.83	0.00	0.11
	1.92	0.10	0.11
Almiron M2	1.28	0.14	0.16
	1.17	0.10	0.20
Nestlé			
Nan H.A.	3.79	0.34	0.00
	4.33	0.39	0.10
Nan	0.94	0.27	0.15
	0.71	0.22	0.12
Alfaré	1.24	1.06	0.48
	1.23	1.03	0.35
Milupa			
Préaptamil	1.52	0.22	0.11
	1.45	0.22	0.13
Aptamil I	2.57	0.58	0.17
	2.41	0.60	0.15
Aptamil II	4.56	0.51	0.47
	4.56	0.47	0.44
Aptamil H.A.	2.38	0.25	0.13
	2.00	0.31	0.09
Nektarmil	2.39	0.23	0.27
	2.90	0.31	0.18
Milumel 1	3.79	0.39	0.32
	3.87	0.44	0.42
Milupa som	0.62	0.35	0.07
	0.63	0.35	0.08
Milupa HN25	6.07	1.73	0.74
	5.82	1.75	0.87
Guigoz			
Guigoz I	2.20	0.25	0.33
	2.34	0.24	0.38
Nativa	1.64		0.25
	1.54	0.56	0.30

* Results are expressed in $nmol \cdot mL^{-1}$ of milk diluted as indicated by the manufacturer. Each value is the mean of two measurements. Each formula is sampled twice.

maturation in the rat also seems to be accelerated by ingestion of spermine. Increased amounts of proteolytic enzymes could be secreted in unweaned rats receiving this polyamine orally, a process that would increase hydrolysis of proteins in chyme. Such an effect would increase the degradation of potentially allergenic substances. When added to a decrease in permeability to macromolecules, it would diminish the amount of allergens reaching the intestinal submucosa and thus would allow a better maturation of the immune system in susceptible children.

Of course, the best way to prove our suggestions would be to increase the amount of polyamines in infant formulas and to prove that newborn children who receive such powdered milks are less frequently subject to symptoms of allergy than newborn children fed with infant formulas currently sold on the Belgian market. We hope to be able to undertake these experiments and the necessary epidemiologic study in the near future.

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