# Fatty Acid Composition of French Infant Formulas with Emphasis on the Content and Detailed Profile of *trans* Fatty Acids

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**ABSTRACT:** The aim of the present study was to identify and quantitate *trans* isomers of C18 fatty acids in some French infant formulas. Twenty powdered infant formulas were purchased in pharmacies and supermarkets in order to assess their *trans* mono- and poly-unsaturated fatty acids content. The fatty acid profiles were examined using methyl and isopropyl ester derivatives. The combination of gas–liquid chromatography, high-performance liquid chromatography, and silver nitrate thin-layer chromatography was needed to describe the detailed fatty acid compositions of the samples, including *trans* isomers of unsaturated C18 fatty acids. All the samples contained *trans* isomers of C18:1 acid (mean level 1.97 ± 0.28% of total fatty acids), with vaccenic acid being generally the major isomer (15 out of 20 samples), thus indicating the origin from bovine milk. All the formulas also contained various isomers of linoleic and α-linolenic acids, but at lower levels. *Trans* PUFA isomers are the same as those present in deodorized oils. In conclusion, all the infant formulas analyzed in this study contained some *trans* fatty acids, including isomers of essential fatty acids. This should be taken into account in the dietary intake of the newborn. *JAOCS 73*, 1595–1601 (1996).

**KEY WORDS:** Distribution pattern, essential fatty acids, infant formulas, *trans* fatty acids.

Geometrical isomers of unsaturated fatty acids are formed during various processes, including biohydrogenation in the rumen (1,2), heat treatments of fats and oils (3,5), and catalytic hydrogenation (6). The biohydrogenation leads to "natural" forms of *trans* isomers of oleic acid. The position of the *trans* double bond varies along the carbon chain, but it is mainly located at the Δ11 position (vaccenic acid) (7). On the other hand, geometrical isomers of 18 carbon polyunsaturated fatty acids (PUFA) are formed during industrial processes, including deodorization and hydrogenation of oils. Both of these processes induce the formation of geometrical isomers of α–linolenic acid (18:3n-3) and linoleic acid (18:2n-6) (8,9). The main isomers formed after heat treatments are the 18:3 Δ9*cis*, 12*cis*, 15*trans*, 18:3 Δ9*trans*, 12*cis*, 15*cis*, 18:3 Δ9*trans*, 12*cis*, 15*trans*, 18:2 Δ9*cis*, 12*trans*, and 18:2 Δ9*trans*, 12*cis*. Catalytic hydrogenation leads to the formation of some of these 18:2 isomers and to positional and geometrical isomers of linoleic acid (9), but also to the formation of *trans*-18:1 fatty acids, with a different localization of the *trans* double bond on the carbon chain than that observed as a consequence of biohydrogenation by bacteria in the rumen.

Some PUFA isomers are metabolic precursors of long-chain *trans* PUFA, as illustrated by their occurrence in tissues from rats fed hydrogenated or heated oils. Geometrical isomers of eicosapentaenoic acid (10,11), docosahexaenoic acid (10), and arachidonic acid (12–15) are formed from these *trans* 18 carbons PUFA.

The *trans* long-chain PUFA may alter some physiological functions, such as platelet aggregation (16–18), electroretinographic response (19). Additionaly, *trans*–18:3 fatty acids may interact with the conversion of linolenic acid at the Δ6 desaturation step (19,20) and the conversion of linoleic acid into arachidonic acid at the Δ5 desaturation step (21). *Trans*- 18:2 fatty acids also alter the conversion of linoleic acid (12,15,22).

Infant formulas are widely used as substitutes for human milk, although the latter is considered as the best food supply for young infants (23,24). The dietary intake of PUFA is of major importance in relation to the nervous tissue development (25). As the only dietary intake of the newborn is milk, it is of particular interest to quantify the intake of these unusual fatty acids by the newborn. Moreover, adverse effects of *trans* fatty acids were suggested in some infant studies (26), and *trans*-18:1 fatty acids are suspected to have deleterious effects on the lipoprotein balance in men (27). In this context, it is important to determine the levels and types of *trans* fatty acids present in infant formulas and to compare these data with those for human milk (28–31).

## EXPERIMENTAL PROCEDURES

*Formulas.* Twenty powdered infant formulas were purchased in supermarkets and pharmacies in France. The different formulas reflected most of the consumption and included formu-

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las for premature (two samples), for infants from birth to 5-months-old (nine samples) and for infants 6–12-months-old (eight samples). One additional formula for infants 10–12-months-old and more was also included in this study.

*Lipid extraction and esterification of fatty acids.* Total lipids were extracted according to Folch *et al.* (32) from reconstituted powdered formulas.

To analyze short chain and *trans* 18:1 fatty acids, isopropyl esters (FAIPE) were prepared as previously described (33,34). Briefly, after evaporation of the solvent to dryness under a nitrogen stream, hexane, isopropanol, and concentrated sulfuric acid were added to the lipid extract. The reaction was allowed to proceed by refluxing in a boiling water-bath for 1 h. FAIPE were extracted three times with hexane in the presence of an aqueous NaCl solution. This procedure allows quantitative recovery of the shorter fatty acids (33). The FAIPE were then analyzed by gas–liquid chromatography (GLC), using a Carlo Erba (Fisons Instruments, Arcueil, France) 4130 chromatograph fitted with a split injector and a flame-ionization detector. The detector and the injector were maintained at 250°C. Helium was used as a carrier gas (inlet pressure, 100 kPa, flow rate: 1 mL · $min^{-1}$). Separations were performed on a CP Sil 88 fused-silica capillary column (50 m × 0.25 mm i.d., film thickness: 0.20 μm; Chrompack, Middelburg, The Netherlands). The column was operated at 65°C for 6 min, and the temperature was then increased at a rate of 5°C · $min^{-1}$ up to 200°C and left at this point until the end of analyses (elution of C22:6n-3). Quantitative analyses were performed with an SP4290 integrator (Spectra Physics, San Jose, CA).

FAIPE were further fractionated by thin-layer chromatography (TLC) on silica-gel plates impregnated with silver nitrate. Precoated plates (DC-Vertigplatten Kieselgel H; Merck, Darmstadt, Germany) were dipped in a solution of $AgNO_3$ in acetonitrile (5%, wt/vol) for 20 min, air-dried, and activated at 105°C for 20 min. The developing solvent was a mixture of hexane and diethyl ether (90:10, vol/vol). At the end of the chromatographic runs, the plates were briefly air-dried and sprayed with an ethanolic solution of 2′,7′-dichlorofluorescein (0.2%, wt/vol). The bands corresponding to the saturated and *trans* monounsaturated fatty acids were visualized under ultraviolet light and scraped off together from the plates. This procedure allows use of endogenous C16:0 and C18:0 as internal standards (34) for the quantification of *trans* monounsaturated fatty acids. FAIPE were extracted with methanol and hexane in the presence of an aqueous solution of NaCl (35). After evaporation of the solvent to dryness under nitrogen, FAIPE were dissolved in hexane and analyzed by GLC. The GLC conditions were the same as above, except that the analyses were performed isothermally at 160°C. Identification of peaks was made by co-injection of the *trans* 18:1 fraction with individual synthetic *trans* 18:1 acids having their *trans* double bonds between C5 and C15.

*Analysis of long-chain fatty acids and* trans *PUFA isomers.* Total lipids were also transesterified using boron trifluoride in methanol (14 %, wt/vol) (36). Fatty acid methyl esters (FAME) were extracted with hexane in the presence of distilled water. They were analyzed by GLC, using a Carlo Erba HRGC 5300 chromatograph, fitted with an automatic on-column injector, a flame-ionization detector, and a DB Wax (J&W Scientific, Rancho Cordo, CA) fused-silica column (30 m × 0.32 mm i.d., film thickness: 0.50 μm). Helium was used as a carrier gas. FAME (from C16 to C22) were identified by comparison with commercial standards and quantitated using the Winer on Windows software (Spectra Physics, San Jose, CA). FAME (20 mg) were also fractionated by reversed-phase isocratic high-performance liquid chromatography (HPLC). A Nucleosil C18 (Shandon, Eragny, France) column (25 cm × 10 mm i.d.) was used for the purification of the C18:2 and C18:3 fractions. The elution was performed with a mixture of acetonitrile and acetone (9:1, vol/vol) at a flow rate of 4 mL · $min^{-1}$. The C18:2 and C18:3 fractions were then analyzed by GLC, using a Hewlett- Packard (Palo Alto, CA) 5890 serie II chromatograph, fitted with a split/splitless injector, a flame-ionization detector and a BPX 70 (SGE, Melbourne, Australia) column (50 m × 0.25 mm i.d., film thickness: 0.25 μm). Helium was used as a carrier gas. Linoleic and α-linolenic acid geometrical isomers were identified by comparison of their equivalent chainlengths (37) with those of synthetic standards (38,39).

## RESULTS AND DISCUSSION

The fatty acid compositions of the different types of formulas (prematures, 0–5 mon, 6–10 mon, up to 10 mon) are presented in Table 1. Except for formulas for prematures which are particularly rich in short-chain fatty acids, the major fatty acids are palmitic and oleic acids. The linoleic acid content varies between 8.89 and 17.54%, except for sample D1 (1.33%), which is available for older infants (>10 mon). The α-linolenic acid level is about 1% of total fatty acids (range 0.52–1.63%). *Trans*-18:1 isomers represent between 0.2 and 4.3% of total fatty acids in the formulas, whereas the *trans* PUFA content varies between 0.04 and 1.01% of total fatty acids. Figure 1 shows the 18:1 *trans* profile of two typical samples. The upper one had a 18:1 *trans* profile of milk fat (high level of Δ16 *trans* isomer and Δ11 *trans* as the major component) whereas the lower part of the figure shows a profile comparable to hydrogenated fat with similar levels of the Δ9, Δ10, and Δ11 isomers. The *trans* fatty acid content is detailed in Table 2. The major *trans* fatty acids are isomers of 18:1, with various positional isomers, ranging from 18:1 Δ6*trans* to 18:1 Δ16*trans*. The two mono*trans* isomers of linoleic acid (18:2 Δ9*cis*, 12*trans* and 18:2 Δ9*trans*, 12*cis*) and several 18:3 isomers (Fig. 2) were found in all the samples. Some differences in the content in each 18:1 *trans* isomers are observed.

Figure 3 shows the degree of isomerization (DI) of 18:2 and 18:3 fatty acids in the 20 samples which were included in this study. The DI for 18:3 can reach more than 20% (two samples) whereas the DI of linoleic acid is generally lower than 5% (except in sample A1).

**TABLE 1**
**Fatty Acid Profile of Infant Formulas[a]**

| | A1 | A2 | Range B1–B9 | Range C1–C8 | D1 |
|---|---|---|---|---|---|
| 4:0 | 1.22 | 1.26 | <0.01–2.81 | 0.01–2.46 | 3.59 |
| 5:0 | 0.05 | 0.01 | <0.01–0.13 | <0.01–0.10 | 0.02 |
| 6:0 | 0.90 | 0.94 | 0.21–1.41 | 0.25–1.56 | 2.41 |
| 7:0 | 0.01 | 0.02 | <0.01–0.01 | <0.01–0.01 | 0.02 |
| 8:0 | 18.60 | 12.83 | 0.82–4.95 | 0.97–3.25 | 1.45 |
| 9:0 | 0.03 | 0.13 | <0.01–0.01 | <0.01–0.02 | 0.02 |
| 10:0 | 14.04 | 9.37 | 1.35–3.65 | 1.18–1.89 | 3.31 |
| 11:0 | 0.02 | 0.04 | <0.01–0.03 | <0.01–0.03 | 0.05 |
| 12:0 | 1.56 | 3.25 | 2.04–13.31 | 2.55–12.01 | 3.66 |
| 13:0 | 0.02 | 0.04 | <0.01–0.05 | 0.01–0.04 | 0.08 |
| *iso* 14:0 | 0.05 | 0.04 | <0.01–0.13 | <0.01–0.05 | 0.13 |
| 14:0 | 3.86 | 5.36 | 4.85–9.10 | 4.71–7.09 | 12.05 |
| *iso*15:0 | 0.09 | 0.08 | <0.01–0.24 | <0.01–0.43 | 0.31 |
| *ante iso* 15:0 | 0.17 | 0.13 | <0.01–0.44 | 0.01–0.33 | 0.46 |
| 15:0 | 0.35 | 0.44 | 0.03–0.97 | 0.06–0.99 | 1.14 |
| *iso*16:0 | 0.07 | 0.09 | <0.01–0.24 | <0.01–0.18 | 0.24 |
| 16:0 | 14.55 | 18.57 | 19.07–25.46 | 20.71–26.32 | 33.29 |
| *iso*17:0 | 0.09 | 0.15 | 0.01–0.32 | <0.01–0.23 | 0.38 |
| *ante iso* 17:0 | 0.01 | 0.20 | <0.01–0.48 | 0.01–0.49 | 0.49 |
| 17:0 | 0.14 | 0.24 | 0.04–0.49 | 0.05–0.51 | 0.59 |
| 18:0 | 5.29 | 5.28 | 4.19–11.55 | 5.11–12.34 | 8.85 |
| 10:1 | 0.10 | 0.13 | <0.01–0.19 | <0.01–0.20 | 0.35 |
| 14:1 | 0.28 | 0.43 | <0.01–0.90 | 0.01–0.40 | 0.99 |
| 16:1 | 0.53 | 0.60 | 0.07–1.54 | 0.11–1.43 | 1.35 |
| 17:1 | 0.09 | 0.12 | 0.02–0.30 | 0.02–0.06 | 0.28 |
| 18:1*c* | 18.02 | 19.96 | 19.70–29.97 | 22.66–29.12 | 16.75 |
| 18:1*t* | 1.46 | 1.02 | 0.21–2.53 | 0.23–4.29 | 2.99 |
| 20:1 | 0.29 | 0.44 | 0.16–0.74 | 0.15–0.78 | 0.15 |
| 18:2*t* | 0.85 | 0.14 | 0.17–0.35 | 0.10–0.51 | 0.01 |
| 18:2*c* | 15.73 | 15.78 | 8.89–17.54 | 10.24–17.45 | 1.33 |
| 18:3*t* | 0.16 | 0.11 | 0.06–0.19 | 0.02–0.23 | 0.03 |
| 18:3 | 0.52 | 1.47 | 0.57–1.63 | 0.76–1.46 | 0.75 |
| conjugated 18:2 | 0.34 | 0.37 | 0.15–1.19 | 0.17–1.01 | 0.97 |
| Saturates | 61.12 | 58.47 | 47.43–58.22 | 51.12–56.79 | 72.44 |
| Monoenes *cis* | 19.31 | 21.24 | 22.10–31.44 | 25.49–30.37 | 19.87 |
| Polyenes *cis* | 16.25 | 17.25 | 10.02–18.88 | 11.44–18.91 | 2.08 |
| P/S ratio | 0.27 | 0.30 | 0.17–0.37 | 0.20–0.36 | 0.03 |

[a]A: formulas for prematures; B: formulas for 0–5-month-old infants; C: formulas for 6–10-month-old infants; D: formula for up to 10 months infants. Results are expressed as percentages of total fatty acids. *c*, *cis*; *t*, *trans*; P/S, polyunsaturates/saturates ratio.

In the present study, we have looked at the fatty acid profile of 20 French infant formulas. As shown in Table 1, about 55% of total fatty acids are saturated; *cis* monounsaturated fatty acids range from 25–30% whereas the total PUFA content (*cis* isomers only) is generally close to 15%. These data are quite similar to those reported some years ago by Jensen *et al.* (40), but slightly different from U.S. liquid formulas which were shown to contain about 45% of saturates and 30% of PUFA (41). German formulas were also generally with lower levels of saturates (42).

Recent recommendations suggest a 18:2/18:3 ratio of about 10 (range 5–15) (43,44). In our study, only 15 out 20 samples meet this criteria, when only the *cis* isomers are considered. Sample D is different because it is composed of pure bovine milk fat, as indicated by the manufacturer and evidenced by our analysis. But four formulas, including A1 (for prematures) presented a too high 18:2/18:3 ratio. The consequences of such compositions of dietary fats for the newborn might be questionned, particularly in relation to the central nervous system and retinal development (24), because large amounts of 18:2n-6 may interfere with the conversion of 18:3n-3 into EPA and DHA, the latter being absent from the formulas, whereas it is a normal component of human milk.

The present data clearly indicate that powdered infant formulas marketed in France contain some *trans* fatty acids, including geometrical isomers of 18:1 resulting from hydrogenation of fats (generally biohydrogenation), but also *trans* isomers of PUFA, resulting from heat treatments of oils, most probably the deodorization (3–5). Because the 18:2n-6 acid is always less than 2% of total fatty acids of bovine milk fat (34), the higher levels present can only be explained by the addition of liquid vegetable oils to the formulas. Milk fat naturally contains some *cis,trans* and *trans,cis* isomers of linoleic acid, but not of α-linolenic acid (34). Our data on

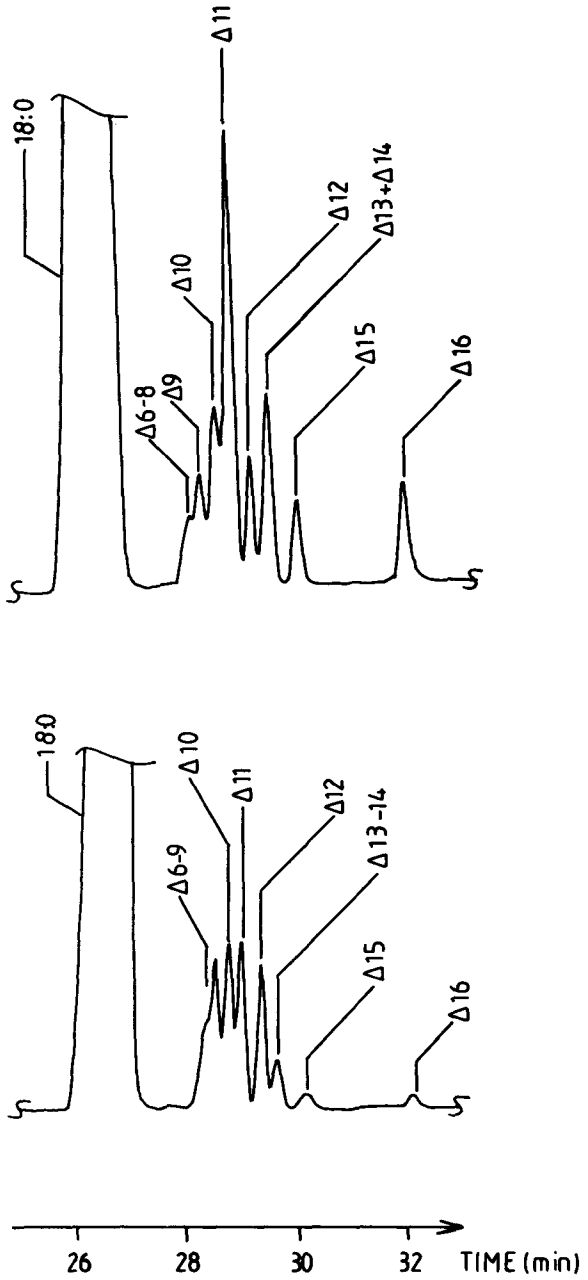


**FIG. 1.** Partial chromatogram of the 18:1 *trans* isomers from sample D1 (A) and sample B4 (B). Column = CP Sil 88 (Chrompack, Middelburg, The Netherlands).

*trans* PUFA are similar to those described some years ago by O'Keefe *et al.* (41) in liquid formulas marketed in the United States. These authors also reported a DI value of 18:3n-3 ranging from 2.6 to 23.5%. In our study, it varies between 3.2 and 23.5%. Although Wolff (45) has shown that in the study by O'Keefe *et al.* (41) the α-linolenic DI was overestimated, the data agree quite well. Similarly, the DI of 18:2 is always less than 5.1% (sample A1) whereas O'Keefe *et al.* (41) reported a maximum value of 2.4%. The total *trans* content varies between 0.43 and 5.04 of total fatty acids, which is lower than recommended by the Commission of the European

**TABLE 2**
***Trans* Fatty Acid Content of Infant Formulas[a]**

| | A1 | A2 | Range B1–B9 | Range C1–C8 | D1 |
|---|---|---|---|---|---|
| 18:1Δ6–Δ9*t* | 0.14 | 0.15 | 0.08–0.29 | 0.09–0.63 | 0.32 |
| 18:1Δ10–Δ11 | 0.73 | 0.49 | 0.04–1.78 | 0.05–2.48 | 1.64 |
| 18:1Δ12–Δ16 | 0.59 | 0.38 | 0.09–1.15 | 0.09–1.18 | 1.03 |
| 18:2Δ9*c*,12*t* | 0.47 | 0.08 | 0.08–0.19 | 0.07–0.33 | 0.01 |
| 18:2Δ9*t*12*c* | 0.38 | 0.06 | 0.08–0.16 | 0.03–0.29 | <0.01 |
| 18:3Δ9*t*,12*c*,15*c* | 0.06 | 0.04 | 0.03–0.10 | 0.01–0.10 | 0.01 |
| 18:3Δ9*c*,12*t*,15*c* | 0.01 | 0.01 | <0.01–0.02 | <0.01–0.02 | 0.01 |
| 18:3Δ9*c*,12*c*,15*t* | 0.07 | 0.06 | 0.04–0.10 | 0.01–0.12 | 0.02 |
| 18:3Δ9*t*,12*c*,15*t* | 0.02 | <0.01 | <0.01–0.01 | <0.01–0.01 | <0.01 |

[a]A: formulas for prematures; B: formulas for 0–5-month-old infants; C: formulas for 6–10-month-old infants; D: formulas for up to 10 month infants. Results are expressed as percentages of total fatty acids.

Communities (44) and more recently by Carrol (46). A possible harmful effect on essential fatty acid metabolism and growth in humans has been suggested (25,46). Moreover, it is important to note than when a newborn ingests *trans* PUFA, these isomers may interact with the metabolism of "normal" *cis* PUFA, as indicated by *in vitro* results (18,22) on the conversion of long-chain PUFA precursors. Now, the recommendations for total *trans* fatty acids are less than 4% of total fatty acids (47). Therefore, 3 out of the 20 French formulas examined do not meet this criteria.

With very few exceptions, the infant formulas contain bovine milk fat, evidenced by their butyric acid content. For example, sample D1 has a typical profile of pure cow milk fat, with no other oil added. One may add that this fat has a composition corresponding to milk collected in winter (34). In such a formula, the *trans*-18:1 acid content may vary with the season, with a maximum of *ca.* 5–6% in spring (34). Medium-chain fatty acids are incorporated in several formulas, particularly A1 and A2 which are formulas for prematures. Enrichment with 12:0 acid (from coconut or palm kernel) is easily detectable, because in milk fat, the ratio 12:0/10:0 is a constant which equals 1.13 or 1.16 depending on the origin (34). Such ratios are effectively found in samples B1 and D1, but in all the other formulas for term infants where this ratio is much higher.

Considering the distribution profiles of *trans*-18:1 acids in the formula lipids, they are generally typical of bovine milk fat, with vaccenic acid being the major isomer. This would indicate that in most instances, no partially hydrogenated oils are added.

The 18:2 Δ9*cis*, 12*trans* is generally present at a higher level than 18:2 Δ9*trans*, 12*cis*. This might be related to the content in refined oils incorporated in the formulas. Similarly, some 18:3 *trans* isomers are found, but at lower levels. Total *trans* PUFA vary between 0.02 and 0.22% of total fatty acids.

In conclusion, the present data show that all the infant formulas included in this study contain *trans* isomers of oleic acid and PUFA. It is interesting to note that the mean value determined for *trans*-18:1 in infant formulas (1.97 ± 0.28%) is similar to that found in the milk (1.91 ± 0.20) from French lactating women (31). On the other hand, the mean levels of *trans* PUFA

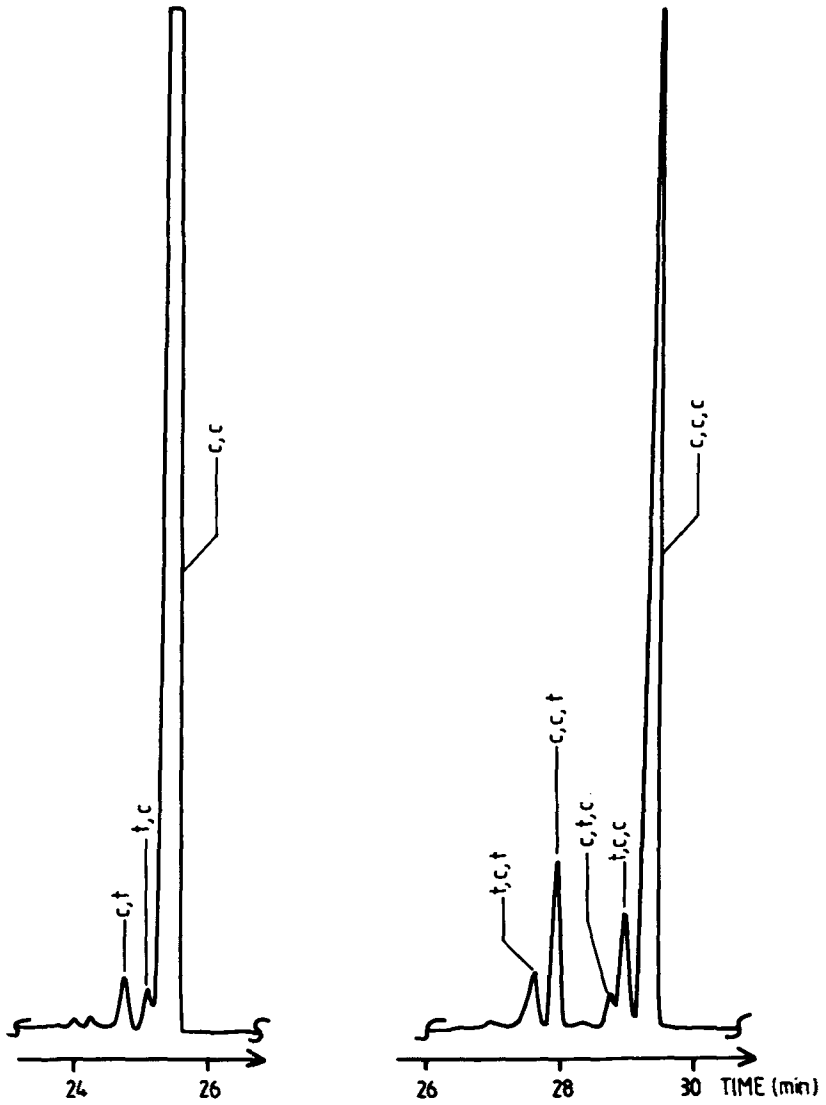


**FIG. 2.** Partial typical chromatogram of the 18:2 isomers (A) and 18:3 isomers (B). Column = BPX 70 (SGE, Melbourne, Australia).

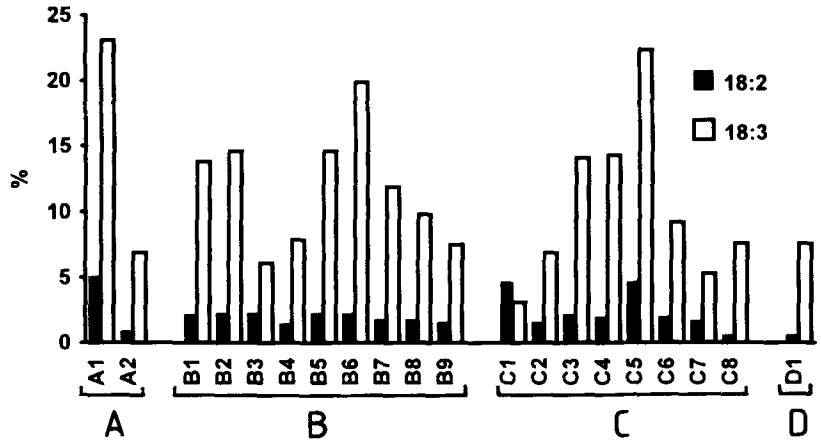


**FIG. 3.** Degree of isomerization (%) of 18:2 (■) and 18:3 (□) fatty acids in formulas for prematures (A), 0–5-month-old (B), 6–10-month-old (C), and 10–12 month-old (D) infants.

were lower in human milk. The respective origins of these *trans* fatty acids are bovine milk fat and deodorized vegetable oils. Even if the total levels of *trans* fatty acids are quite low, they might be taken into account in the dietary intake of fatty acids by the newborn, particularly for prematures.

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