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Studies on changes in vitamin-E and fatty acids of neonatal serum

Correlation of data to diet, age and development.

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With 3 figures and 5 tables

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Introduction

Unfortunately, the composition of mothers' milk is only of limited value as a guide to the optimal lipid content of the diet in infancy, because the lipids in the milk depend upon the lipids in the mother's diet. The lipids in the milk are either synthetized in the mammary gland (33, 34) or transferred to the milk from the fraction of the serum triglycerides found in the pre-ß- and ß-lipoproteins, which are precipitable with dextran sulphate (8). Furthermore, the content of essential fatty acids (linoleic acid) in the milk depends upon the mother's intake of these acids.

The lipid fractions which determine the composition of the biomembranes include both the essential fatty acids as well as the atheromatogenic fatty acids (saturated fatty acids). In the neonatal period, however, the concentration of these fatty acids in serum has been shown to change in a certain fixed pattern, independent of the diet.

Previous studies (3, 6, 39, 41) have shown that infants are born with a high serum level of arachidonic acid and low linoleic acid as compared to adults, but after the first week of life the pattern approaches that of adults and after this period, the concentration of linoleic acid and cholesterol in serum will, to a large extent, depend upon the linoleic content in the diet (6, 26, 30, 38, 42, 43, 46). Thus, in a previous study (6), it was shown that the relative serum concentration of linoleic acid could increase to 32%, or twice normal values, if the baby was given a formula where the milk-fat was substituted by polyunsaturated vegetable oils.

The polyunsaturated fatty acids may however, undergo peroxidative changes (cf. the review by BIERI (7)) especially in conditions of relative vitamin-E deficiency, and as newborns possess low vitamin E level (36), we have found it relevant to elucidate changes in the fatty acid pattern in the neonatal period in more detail, and relate them to possible changes in vitamin-E serum level in the same period. The changes found have also been related to diet, and birth weight.

Materials and methods

Patient material

The sera studied all originated from the neonatal ward or the obstetrics departments, Rigshospitalet, Copenhagen. The sampling was performed 1 to 2 hours after the morning meal. At birth blood was taken from the mothers by puncture of a cubital vein and from the newborns as cord blood. After birth, capillary blood was used. The blood was centrifuged immediately after its coagulation and the samples were hereafter deepfrozen (—20°) until analysis. The fatty acid content and the content of vitamin-E were assayed maximally 8 days after sampling.

Data from 27 children with a birth weight above 2500 g were compared with data from 6 children with a birth weight below 2500 g. All children were healthy and thriving well, they were fed mothers milk supplemented with „half skimmed milk", Eldon, (E).

Chemicals

Unless otherwise stated, the chemicals, of highest obtainable purity, were obtained from British Drug Houses, Dorset, England.

Extraction and methylation of fatty acids

The lipids were extracted from 200 to 500 µl serum and from 500 µl milk with 20 vols. chloroform + methanol (2+1 (v/v) and washed with the theoretical upper phase a. m. FOLCH et al. (21). The methylesters of the fatty acids were hereafter made directly from the chloroform phase by transmethylation with HCl in superdry methanol (12). The methylesters were extracted from the reaction mixture with di-ethyl-ether and concentrated to about 5% (w/v) mixture by evaporation at 40° below nitrogen.

Gaschromatography

The fatty acid pattern was estimated on 0.5 µl methylester sample in Perkin Elmer gas-chromatographs, Model F–7 and F–11, equipped with a flame ionization detector and 2 m long columns of 20% (w/w) di-ethyleneglycolsuccinate on Chromosorb W (80–100 mesh) (Applied Science Labs. Pennsylvania, USA), or in order to identify the unsaturated fatty acids, on 1 m columns of 15% (w/w) Apieson L on Chromosorb (80–100 mesh). Nitrogen was used as carrier gas and the temperature of the injection block was 300° and of the columns 195° with the first mentioned type of column, but 150° in the last mentioned type. The fatty acids were identified either by means of methylester standards from The Hormel Institute (Minnesota, USA), or by log-plotting and estimation of retention volumes (31). The analytical error was estimated to be 5% for palmitic acid but maximal 20% for longchained acids such as arachidonic acid.

Quantitative estimation of total fatty acids

The total content of fatty acids in serum, milk and milk-products was assayed by the cupri-reaction (14,20): 300 µl serum, 500 µl mothers milk or about 10 to 50 mg freeze dried milkpowder were extracted for 24 hours with 10 vols. chloroform + methanol (2+1, v/v), the chloroform phase was then evaporated to dryness below nitrogen at 40°. 2 ml 1 M KOH in 50% (v/v), aqueous ethanol was added. After saponification at 86° for 40 min. in glass stoppered glass tubes, concentrated HCl was added until the pK-value of methyl-red. Hereafter, the fatty acids were extracted a. m., Dole and Meinertz (16), with 5 ml extraction-mixture (4 vols. isopropanol + 1 vol. heptane + 0.1 vol. 1 N H_2SO_4). After shaking and extraction for 10 min. at 22°, 2 ml heptane and 3 ml distilled water were added. 2 ml of the heptane phase was isolated and cupri-reagent was added (10 vols. 6.5% (w/v) cuprinitrate trihydrate dissolved in distilled water + 9 vols. 1 N triethanolamine + 1 vol. 1 M acetic-acid). The mixture was shaken for 2 min. whereby cupri-soaps were formed. The water phase was eliminated. 1 ml of the heptane phase was isolated and 500 µl reaction mixture for detection of cupriions was added (this solution contained : 0.1 g Na-diethyl-dithio-

carbamate dissolved in 100 ml. sec. butanol and three drops of formaldehyde (this reagent is stable for 1 week)) (17, 20). The amount of cupri-diethyl-dithiocarbamate was estimated photometrically at 440 nm. With standard solutions of palmitic acid (99% pure Applied Science Labs. Pennsylvania, USA), a linear relationship was found between the extinction and the palmitic acid content as long as the amount of this acid was between 0.2 and 1.0 mg palmitic acid in the reaction mixture. The recovery from serum was estimated to 90%. During the systematic study of the method, it was found necessary to add a few drops of formaldehyde to the reaction mixture, because traces of cyanide ions in the fatty acid standard, through complex formation with the cupriions, would decrease the extinction value.

Quantitative estimation of vitamin-E

The vitamin-E content of serum and mothers milk and milk-powders was estimated as the amount of ferri-ions, which in optimal assay conditions are reduced to ferro-ions by means of vitamin-E. The assay conditions involved addition of surplus of ferri-ions, and the remaining ferri-ions after the reduction had been fulfilled were estimated photometrically by complex-formation with Batophenantrolin (4,7–diphenyl-1-10-phenantrolin) (19). All organic solvents used in this method were protected against photochemical changes by storage in dark flasks covered on the outside with aluminium foil. The proteins in 400 µl serum or milk were precipitated with 400 µl 96% (v/v) ethanol. Hereafter 400 µl xylene was added and after shaking at 22° for 10 min., the mixture was centrifuged (2000 g, 10 min.) and 200 µl of the xylene phase was mixed with 100 µl Batophenantrolin (400 mg/100 ml 100% ethanol) and 100 µl $FeCl_3 3H_20$ (60 mg/100 ml 100% ethanol). The sequence of additions of the reagents is, as stated by FABIANEK et al. (19), critical. After shaking for 20 min. the extinction was assayed at 536 nm. All glassware used in this method was rinsed in the detergent RBS 25 (Bie & Berntsen, Ltd., Copenhagen, Denmark), followed by cleaning in 3 M HCl and five washings in distilled water. A standard solution in xylene of alpha-tocopherol (1 IU/mg, Serva, München, Germany) showed a linear relationship between extinction and vitamin-E concentration between 0.002 and 1.0 $\times 10^{-2}$ mg alpha-tocopherol in the reaction mixture. Recovery of alpha-tocopherol from normal human serum was estimated at 96%.

In the artificial diet for babies derived from milk powder, the vitamin-E was determined in 500 mg material, which was extracted with 5 ml ethanol + 5 ml xylene for 24 hrs. 400 µl of the xylene phase was isolated and further 400 µl ethanol + 400 µl distilled water + 400 µl xylene were added. 200 µl of the xylene phase was used for the vitamin-E assay. (vide supra).

Statistical evaluation

The results were treated statistically (24) by calculation of the standard deviation for values obtained at different ages after birth. Furthermore, the „Skewness" was estimated, in order to secure „normal distributation", and the regression curves relating the values at different times after birth to the age in days were estimated. It was finally calculated whether the slopes of the regression curves were different from zero. All statistical calculations were made on an Olivetti computer. (Model Programma no 101) with the computer programs nos. 1.50, 1.52 and 3.40 (47).

Results

The serum content of vitamin-E and fatty acids in newborns

Table 1 demonstrates the mean values for the serum vitamin-E level in the first week of life. The mean value for the vitamin-E level in newborns with a birth weight above 2500 g, fed mothers milk supplemented with E is higher than in children having a birth weight below 2500 g, but great individual differences occur (cf. the range values). The cord blood at birth exhibits values which are much lower than in adults. The vitamin-E level increases during the first week of life (Fig. 1 and table

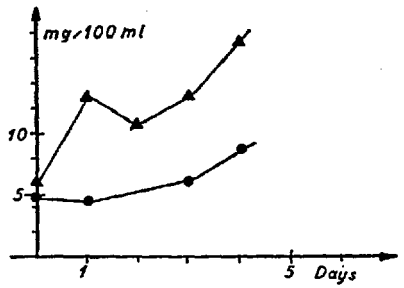

Fig. 1. Vitamin E content of serum from children with birth weight above 2500 g (—▲—▲—▲—▲—) and for children with a birth weight below 2500 g. (—●—●—●—●—). *Ordinate:* Means of E-vitamin content in serum (mg/100 ml). *Abscissa:* Age in days.

Table 1. Concentration of Vitamin E in Human Sera

	mg Vit E/100 ml serum.
Normal Human adult (both sexes). N = 10	1.09 ± 0.23 R: 0.77 — 1.40
Normal Newborns (within one week of age): Diet: Mothers milk supplemented with E. N=36	1.02 ± 0.91 R: 0.11 — 4.35
Newborns (within one week of age). Birth weight ≦ 2500 g N = 4 Diet: Mothers milk supplemented with E.	0.60 ± 0.20 R: 0.32 — 0.96

N = number
R = range of values
E = Eledon Nestle (Half skimmed milk).

Table 2. Concentration of Vitamin E in Human Sera. First day of Life.

	Serum mg Vit. E/100 ml.
Normal Human Adults (both sexes)	1.09 ± 0.23 R: 0.77 — 1.40
Normal Newborns. Birth weight > 2500 g Diet: Mothers milk supplemented with E. Cord serum: N = 12	0.61 ± 0.51
Newborns. Birth weight ≦ 2500 g Diet: Mothers milk supplemented with E. Cord serum: N = 4	0.49 ± 0.21

E = Eledon Nestle (Half skimmed milk).

Table 3. Data of regression analysis of some changes in fatty acid pattern of newborn serum during the first week of life. The data were analysed in two periods, namely the first two days (the initial period with change from transplacental to peroral food intake), and the following four days. The daily changes in percentage of fatty acid and its significance are given.

Fatty acid:	$C_{16:0}$; Palmitic acid				$C_{16:1}$; Palmitoleic acid				$C_{18:0}$; Stearic acid			
Time interval:	0-2 day		3–6 day		0–2 day		3–6 day		0–2 day		3–6 day	
Data:	Daily Change	P-Level	Daily Change	P-Level	Daily Change	P-Level	Daily Change	P-Level	Daily Change	P-Level	Daily Change	P-Level
Birth weight above 2500 g	+4.7	0.1%	—0.81	20%	+0.39	not sign.	+0.22	not sign.	—0.53	2.5%	+1.00	0.1%
Birth weight below 2500 g	—4.0	0.1%	—2.6	5%	—0.96	not. sign.	—1.0	10%	+1.8	15%	+0.43	10%
	$C_{18:1}$; Oleic acid				$C_{18:2}$; Linoleic acid				$C_{20:4}$; Arachidonic acid			
Birth weight above 2500 g	+5.16	0.1%	+2.5	2%	—3.72	0.1%	+0.72	5%	—0.77	10%	—0.87	2%
Birth weight below 2500 g	+5.40	0.5%	+1.8	not sign.	0.09	not sign.	+0.60	not sign.	+0.06	not sign.	+0.52	not sign.
Units:	%/day		%/day		%/day		%/day		%/day		%/day	

2). Due to the great individual differences in the vitamin-E level, the regression curve relating the vitamin-E value at different times after birth to the age in days, did not show a slope which differed from zero in the groups with a birth weight above 2500 g. However, in the individuals with a birth weight below 2500 g the regression curve showed a slope of 0.05 mg vitamin-E/100 ml serum x days, this slope was significantly different from zero on the 5% level. The initial ordinate value was found to be 0.47 mg vitamin-E/100 ml serum for this group.

In a previous work (6) we showed that the content of linoleic acid ($C_{18:2}$) in serum from newborns increased relatively from the second to the seventh day after birth. This change was associated with a decrease in palmitic acid ($C_{16:0}$). In the present paper these studies were continued by gaschromatographic analysis of the fatty acid pattern of serum from children fed mothers milk supplemented with (E) (Fig. 2)., thus also the changes in the first two days of life were elucidated. Fig. 2. shows that the relative content of arachidonic acid ($C_{20:4}$) and stearic acid ($C_{18:0}$) declined in serum throughout the whole period. The content of oleic acid ($C_{18:1}$) increased in the same period. Contrary to this, the serum content of palmitic acid ($C_{16:0}$) increased in the first two days and decreased in the following four days. Linoleic acid ($C_{18:2}$) in the first two days decreased and increased in the next four days. Most of these changes were significant as shown by regression analysis (24) (cf. table 3). In the present communication it was not possible to obtain sufficient data from the second week of life since the babies and their mothers were sent home after one week of hospitalisation. Data from the second week of life were however, obtainable in the group of premature since these were hospitalised for longer periods. In this group fed mothers milk supplemented with (E), the changes in the two periods mentioned above were often opposite to those found in the group with a birth weight above 2500 g (table 3). Thus apart from the steady increase in oleic acid in both periods, the initial decrease in linoleic acid and increase in palmitic acid was not found. Finally, neither could the fall in arachidonic acid be traced in serum from premature babies.

With regard to the total fatty acid content of newborn serum from children fed mothers milk supplemented with (E) compared to the value of the mothers, it was found that the total fatty acid content was low at birth, and that the content increased nearly significantly as function of age ($p \geqq 10\%$) (Table 4, fig. 2.). However, great individual differences occurred (cf. the SD values in table 4).

Table 4. Total fatty acid content of serum from infants and mothers in the neonatal period.

	mg/100 ml
Total fatty acids of mothers serum at birth.	318.4 ± 50.2
Total fatty acids of cord serum. N = 6 Diet: Mothers milk supplemented with (E).	133.2 ± 19.7
Total fatty acids of infant serum in neonatal period (all data taken together) Diet: Mothers milk supplemented with E. N = 31	166.2 ± 41.7

E = Eledon Nestle (Half skimmed milk).

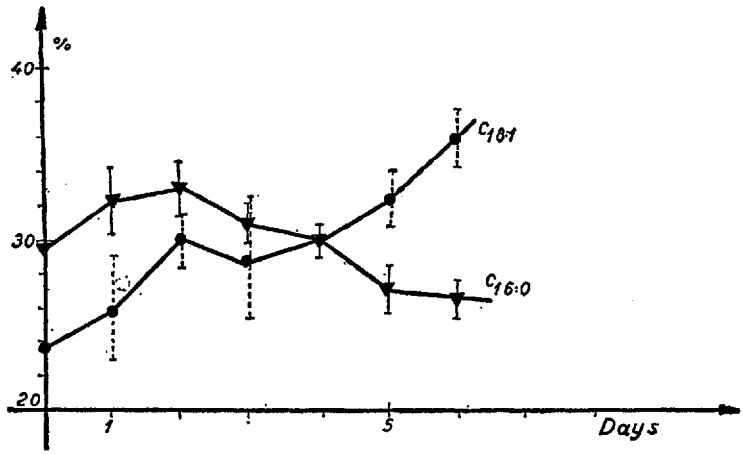

fig. 2a

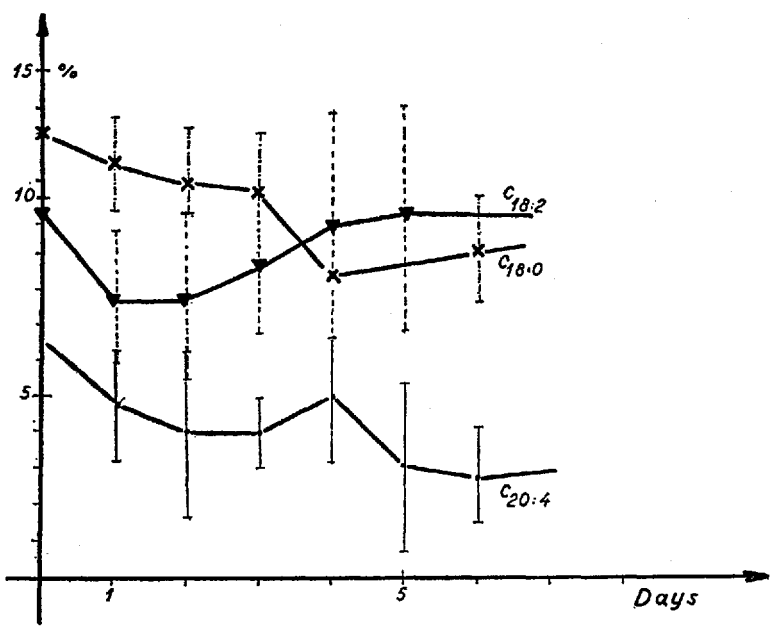

fig. 2b

Fig. 2a and b. The relationship between the mean values at different days after birth of the relative content of palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), linoleic acid ($C_{18:2}$) and arachidonic acid ($C_{20:4}$) and the age (days). *Ordinate:* Means of the relative percentage of the fatty acid. (per cent of total content of fatty acids). *Abscissa:* Age in days.

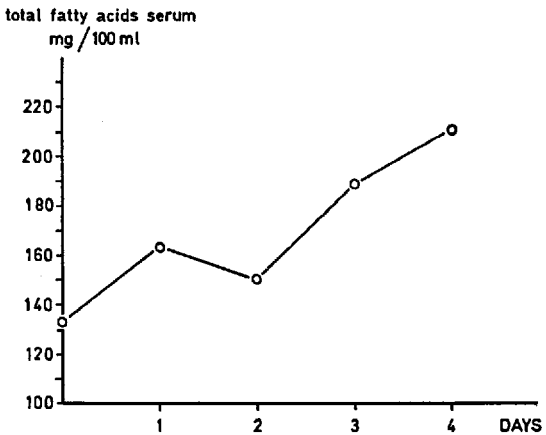

Fig. 3. The relationship between the total fatty acid content of serum in babies during the first days of life and the age in days. *Ordinate:* The mean values of the total content of fatty acids in serum at different ages. (mg/100 ml). *Abscissa:* Age in days.

By regression analysis of the single values involved in fig. 3, a rough linear relationship between the time and fatty acid content could be deduced. The slope of the regression line was b = 7.0 mg fatty acid/100 ml serum × days. This slope was only significantly different from zero on the 10% level. It was calculated that the regression line cut the ordinate at 148.3 mg fatty acid/100 ml serum.

The low serum content of vitamin-E at birth found in the present communication, may be explained by a low transport capability of this vitamin across the placenta (43), and must be connected with the fact that if the proportion between mg alpha-tocopherol and gram of polyunsaturated fatty acids in the diet decreases below 0.6, a danger for relative vitamin-E deficiency may occur (26). This can give rise to weakening of the plasma membrane of erythrocytes and hereby to hemolysis (27, 38). These facts stimulated us to elucidate the fatty acid content of mothers milk in relation to its content of vitamin-E, and to compare the values to those of artificial milk-powders used for feeding newborns.

Studies on the content of fatty acids and vitamin-E in diets of newborns

Table 5 shows the mean values of the fatty acid patterns of mothers milk, as well as the total fatty acid content and the vitamin-E value. Similar data are given for the composition of some of the milk-powders used for feeding newborn infants. It is seen that the relative content of linoleic acid in cows' milk, both whole milk and separated milk, is only half of that found in mothers' milk. A similar pattern of the fatty acid is also seen in the milk-powders unless they have been supplemented by vegetable oils. Table 5 also shows the values of total fatty acid and vitamin-E content of the products studied. It may be seen that although great differences occur in the content of both vitamin-E and polyunsaturated fatty acid, in all cases the ratio: mg-tocopherol/g polyunsaturated fatty acids is above 0.6, a value indicated by Harris & Embree (26) to be the critical lower limit below which, danger of induction of relative vitamin-E deficiency may occur.

Table 5. The table indicates the relative fatty acid pattern (%), the total fatty acid content and the total vitamin-E content of mothers milk, cows milk and derivatives of cows milk, and substitutes of mothers milk.

Fatty acids % of total:	EK N=3	E N=1	P N=3	N N=2	NA N=1	V N=1	M N=2	Ve N=1	Mothers Milk N = 17	Skimmed Milk N = 16	Whole milk N = 16	Butter Milk N = 16
C:12		0.8	0.36 ± 0.46	0.35 ± 0.22	0.5		1.10 ± 0.56					
C:13	1.96 ± 1.35	2.8	3.70 ± 1.01	3.00 ± 0.56	2.6	1.7	2.70 ± 1.83			0.7 ± 0.3	1.3 ± 0.4	0.4 ± 0.1
C:14x*		0.5	0.36 ± 0.31	0.25 ± 0.16			0.70 ± 0.14			0.1	0.40 ± 0.01	0.30 ± 0.01
C:14	3.36 ± 0.87	5.4	5.43 ± 1.82	3.85 ± 1.48	2.8	2.9	5.20 ± 0.56	0.3	1.4 ± 1.2	1.9 ± 0.6	3.3 ± 0.7	2.6 ± 0.7
C:15z*										0.03 ± 0.01	0.10 ± 0.05	0.10 ± 0.01
C:15y*		0.5		0.30 ± 0.22						1.1 ± 0.3	1.30 ± 0.37	1.4 ± 0.3
C:15x*	0.33 ± 0.14	0.1	0.13 ± 0.14			0.7	0.10 ± 0.04		0.45 ± 0.43	1.7 ± 0.5	2.9 ± 0.7	2.5 ± 0.7
C:15	11.73 ± 0.67	12.9	15.16 ± 2.41	11.30 ± 1.13	10.5	10.3	12.60 ± 2.12	0.7	6.1 ± 2.0	10.7 ± 2.7	11.8 ± 3.0	11.6 ± 3.0
C:16z*	1.83 ± 0.31	2 8	2.23 ± 0.20	2.45 ± 0.22	2.3	2.3	2.15 ± 0.22					
C:16y*	1.20 ± 0.26	1.8	1.50 ± 0.26	1.75 ± 0.10	1.7	1.5	1.45 ± 0.36		0.33 ± 0.16			
C:16x*		0.2	0.03 ± 0.00	0.10 ± 0.04		0.1	0.20 ± 0.14				0.6 ± 0.1	0.60 ± 0.13
C:16	35.90 ± 4.35	24.8	32.63 ± 0.94	31.70 ± 1.97	30.3	30.9	28.45 ± 4.45	12.5	24.2 ± 2.8	32.6 ± 8.2	26.1 ± 7.0	30.0 ± 7.0
C:16:1	2.76 ± 0.90	5.4	3.60 ± 0.85	4.50 ± 1.27	5.4	3.7	4.00 ± 0.70		6.5 ± 1.4	2.0 ± 0.7	2.8 ± 0.7	2.2 ± 0.6
C:16:2*	0.36 ± 0.33	1.0	0.53 ± 0.32	0.45 ± 0.64			1.10 ± 0.28					
C:17	0.50 ± 0.26	1.1	0.66 ± 0.00	1.45 ± 0.64	1.9	0.9	0.90 ± 0.42		0.42 ± 0.27	0.60 ± 0.07	0.80 ± 0.25	1.20 ± 0.30
C:Fyt		0.5	0.10 ± 0.07	0.15 ± 0.12		0.3	0.40 ± 0.14		0.23 ± 0.16		0.20 ± 0.07	0.20 ± 0.01
C:18	10.96 ± 0.37	11.2	9.20 ± 2.34	8.35 ± 1.06	7.6	12.1	11.30 ± 1.97	4.3	12.4 ± 1.8	14.2 ± 3.5	12.7 ± 4.2	13.2 ± 4.2
C:18:1	25.36 ± 3.30	24.8	21.10 ± 3.27	22.00 ± 0.70	21.3	27.5	24.10 ± 4.24	35.4	36.3 ± 3.3	29.3 ± 7.0	30.0 ± 7.5	28.7 ± 7.0
C:18:2	3.83 ± 2.19	3.2	3.16 ± 0.64	7.20 ± 6.08	11.5	4.5	3.45 ± 0.78	41.4	10.1 ± 2.4	4.6 ± 1.3	5.0 ± 1.3	4.5 ± 1.1
C:18:3		0.2		0.90 ± 0.70	1.4	0.7	0.25 ± 0.10	5.0	0.55 ± 0.34		0.40 ± 0.05	0.30 ± 0.10
C:20:1									0.18 ± 0.10		0.30 ± 0.10	0.10 ± 0.01
C:20:3											0.10 ± 0.01	0.10 ± 0.01
1. Total fatty acids	0.02 ± 0.00	0.151	0.04 ± 0.00	0.21 ± 0.10	0.17	0.091	0.42 ± 0.17	0.128	665 ± 311[3]	[4]	[4]	[4]
2. E-Vitamin	2.19 ± 0.87	19.34	2.86 ± 0.48	4.93 ± 0.14	5.0	1.08	2.12 ± 0.42	23.57	0.72[3]	0.06 ± 0.02[3]	0.104 ± 0.2[3]	0.08 ± 0.03[3]
Vit. E mg/g PUFA	23.10 ± 7.71	37.7	18.43 ± 4.84	3.90 ± 2.40	3.2	2.3	1.49 ± 0.71	34.3	10.2			

EK = Eledon komplet
E = Eledon
P = Pelargon
N = Nespray
NA = NAN
V = Vitana
M = Mamysan B
Ve = Velactin
Fyt = Fytanic acid
PUFA = Polyunsaturated fatty acids

[1]) = mg/mg DW
[2]) = μg/mg DW x 10^{-2}
[3]) = mg/100 ml
[4]) = Not performed due to high level of volatile fatty acids.
* unidentified fatty acids

Discussion

Previous studies on the fatty acid pattern of serum and organs during the newborn period, have revealed changes as function of time related both to developmental and dietary factors. Thus BERG-HANSEN and CLAUSEN (4) showed in experiments with rats, changes in the fatty acid pattern of the central nervous system during the first two months after birth, a period with the most intensive myelination (1, 10, 11). These changes (4) involved an increasing amount of oleic acid, deposited in phospholipids of the central nervous system but a decrease in the amount of palmitic and arachidonic acid, deposited in the same lipid fractions. These changes were associated with a significant increase in the amount of ethanolamine-phosphatides but a decrease in the amount of choline-phosphatides. The changes of the central nervous system were, to a great extent, independent of the diet. Similar findings were found in a study of Danish and African autopsy material of human fetuses. In these studies (3, 5) thus involving two different evironmental areas the changes in fatty acids and phospholipids were approximately independent of the diet, probably because the babies are born with deposits of arachidonic acid.

The present paper is an extension of the above-mentioned studies and our recent studies of the fatty acid pattern of newborns (6). Thus it is evident from the present communication, that even within the first two days of life, significant changes occur in the fatty acid pattern of newborns with a birth weight above 2500 g, fed mothers milk supplemented with (E). The changes of this period, where the organism alters its metabolic state, due to a transfer from transplacental to peroral nutrition, do not entirely seem to mirror the changes found in the following four days. The initial decrease in serum linoleic acid may thus be explained by a periferal consumption of this essential fatty acid without a corresponding uptake from the intestinal tract. The increase in linoleic acid in the following days may be explained by an increasing uptake of this essential fatty acid, because a large amount of this fatty acid occurs in mothers milk (30). These data agree with recent investigations (26, 38, 42, 43), thus confirming that the concentration of linoleic acid in serum during childhood depends on the concentration of this fatty acid in the diet. In children with a birth weight below 2500 g the serum changes in the fatty acid pattern do not correspond to those with a birth weight above 2500 g. Probably this discrepancy may be explained by a slower maturation of the enzyme systems. However, it must be stressed that the material within this group is inhomogenous because the birth weights were found within wide limits (1000 to 2400 g).

The low total fatty acid content of newborn serum found in the present paper is in agreement with HERINGS data (28), although our values for the total fatty acid content of the mothers are lower than found by HERING (28). The low total fatty acid content of newborn serum supports the theory that the fetus mainly covers its energy demand by burning carbohydrates (41) and also the theory that fatty acids have difficulty in passing the placenta, probably due to albumin binding in the maternal serum (2, 36). These data also correspond to the demonstration (2, 37) that newborn serum contains low values for unesterified fatty acids. However, the fatty acid pattern of the newborn may also be determined either by a difference in the metabolism of different fatty acids in the placenta, i. e. arachidonic acid (32), or in the velocity of passage of different phospholipids through the placenta (9), or finally by a selectivity in the passage of free fatty acid through the placenta (41). This latter theory is supported by studies of RENKONEN (39) and by our own experiments pre-

viously (6), and in the present communication which shows that the human newborn possesses a relatively large amount of arachidonic acid, and furthermore that both Danish material and material from East Africa (5, 6) revealed a significant correlation between the maternal serum concentration of linoleic acid, and the relative amount of this acid in the cord blood of the newborn. Furthermore, the increasing amount of total fatty acids during the first days of life, demonstrated in the present communication, coupled with the changes in the proportions between the different fatty acids, give rise to either a constant or increasing level of all main fatty acids studied in the present paper. Thus, the total amount of arachidonic acid, even though its content relative to the other fatty acids decreases, in absolute value increases from the first to the fourth day of life from 8.7 to 10.5 mg/100 ml serum. The palmitic acid seems rather constant in the same period, because it possesses a value of 16.3 mg/100 ml at birth and 17.2 mg/100 ml on the fourth day of life. These data support the idea that during the newborn period, an increasing intake of fatty acids occurs through the diet, not only for the supply of energy but also in order to supply enough fatty acids for incorporation in the membranes, e. g. the myelin membranes, and in order to cover the membrane formation related to cellular proliferation during the neonatal period (2, 3, 4, 5, 6).

Even in cases of lowest serum level of vitamin-E combined with the highest absolute concentration of polyunsaturated fatty acids (mainly linoleic and arachidonic acid), the proportion between mg vitamin-E and g polyunsaturated fatty acids seems to be higher than this ratio in serum from normal adults. Thus the danger of peroxidation of polyunsaturated fatty acids does not appear to be greater in newborns than in adults. This agrees with the demontration, also in this paper, that the substitutes of mothers milk which are offered in Denmark do not give rise to the same risk for relative vitamin-E deficiency as some of the products available on the market in USA (13, 29), and these data thus correspond well to clinical experiments that hemolysis in newborns due to vitamin-E deficiency is seldom seen in Europe, but more often encountered in USA (27, 39).

The low level of vitamin-E at birth may be explained by hampered transport across the placenta of this vitamin (15, 18, 47). In the intra-uterine condition, the need for vitamin-E as an anti-oxidant may be lower than in the extra-uterine life, because the arterial and venous oxygen tension is lower in the fetus than after birth (34), whereby the need for vitamin-E qua anti-oxidant must be lower. This theory corresponds to the increasing need for vitamin-E under hyperbaric conditions (22, 35). The changes in the relative content of arachidonic acid and linoleic acid in the newborn do not seem, per se, to give rise to a greater need for vitamin-E than in the adult stage because the sum of these two acids is lower in the newborn than in the adult. On the other hand, the low vitamin-E level at birth may, under pathological conditions e. g. hyperbilirubinemia (17), predispose to hemolysis (23).

Zusammenfassung

Die Fettsäureverteilung und der Gesamtgehalt an Fettsäuren und E-Vitamin im Serum wurde in den Tagen nach der Geburt der Neugeborenen verfolgt und mit den Werten der Mutter und teils mit den Werten der der Kinder angebotenen Nahrung verglichen.

Beim Vergleich der polyungesättigten Fettsäuren mit dem E-Vitamingehalt in der Babykost konnte festgestellt werden, daß mit der Ernährung durch eine europäische Kost kein Risiko für einen relativen E-Vitaminmangel vorliegt und damit normalerweise kein nahrungsbestimmtes Risiko für eine peroxidative Hemolyse vorhanden ist.

Andererseits sind Befunde über einen niedrigen E-Vitamingehalt bei Geburt von Kindern mit Geburtsgewicht unter 2500 g, jedoch ein normaler Gehalt bei Kindern mit Geburtsgewicht über 2500 g festgestellt worden.

Das erste Verhältnis muß durch einen herabgesetzten Transport des E-Vitamines durch die Placenta erklärt werden. Der Fettsäuregehalt im Serum in der Geburtsperiode konnte beträchtliche Änderungen aufweisen. So ist der Gesamtgehalt an Fettsäuren im Geburtsaugenblick mehr wie zweimal so niedrig wie der der Mutter. Der Arachidonsäuregehalt ist höher, während der Linolsäuregehalt niedriger ist als der der Mutter. Innerhalb der ersten Tage nach der Geburt sank der Linolsäuregehalt relativ im Serum, während der Gehalt an Palmitinsäure stieg. In den folgenden vier Tagen stieg die Linolsäuremenge, während die Palmitinsäuremenge sank. Die Ölsäure stieg relativ während der ganzen Periode hindurch, während Stearinsäure und Arachidonsäure absanken.

Obenerwähnte Änderungen in der Fettsäureverteilung wurden durch eine Regressionsanalyse statistisch behandelt und in Beziehung zu Kost, und Geburtsgewicht gesetzt. Zum Schluß wurden die Ergebnisse auf Grundlage der vorliegenden Literatur über die Physiologie der Geburtsperiode besprochen.

Summary

The fatty acid pattern, the total content of fatty acids and Vitamin-E of serum was assayed in newborns during the first weeks after birth. The data obtained were compared to the data of the mothers and to the newborn's diet.

By relating the total content of polyunsaturated fatty acids to the vitamin-E content of the diet used for feeding newborns in Denmark, it was concluded that normally there was no risk of dietary induced vitamin-E deficiency and hereby no risk of peroxidative hemolysis. On the other hand, a low serum content of vitamin-E was encountered in newborns with a birth weight below 2500 gram. The serum content of vitamin-E was found to be normal in normal newborns with a birth weight above 2500 gram.

The fatty acids of serum revealed pronounced changes during the neonatal period. Thus at birth the arachidonic acid content was higher, but the linoleic acid content lower than in adults. During the first two days of life, linoleic acid decreased and palmitic acid increased. In the following four days linoleic acid increased and palmitic acid decreased relatively. Stearic acid and arachidonic acid decreased and oleic acid increased in the whole period mentioned. The fatty acid findings mentioned were treated statistically by means of regression analysis and related to diet, and birth weight. Finally, the results were discussed on a basis of the literature available on the lipid metabolisn of the neonatal period.

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