Brain Cell and Tissue Recovery in Rats Made Deficient in n-3 Fatty Acids by Alteration of Dietary Fat[1]

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ABSTRACT Rats were fed a purified diet containing either 1.5% sunflower oil [940 mg linoleic acid [18:2(n-6)]/100 g diet; 6 mg α-linolenic acid [18:3(n-3)]/100 g diet] or 1.9% soybean oil [940 mg 18:2(n-6)/100 g diet; 130 mg 18:3(n-3)/100 g diet]. In all cells and tissues examined 22:6(n-3) was lower and 22:5(n-6) was higher in rats fed sunflower oil than in rats fed soybean oil. Levels of 22:4(n-6) and 20:4(n-6) were largely unaffected. Expressed as a percentage of that in soybean oil–fed rats, 22:6(n-3) in sunflower oil–fed rats was as follows: neurons, 49; astrocytes, 47; oligodendrocytes, 10; lung, 27; testes, 32; retina, 36; liver, 35 and kidneys, 45. Ten wk after the change in diet of 60-d-old rats from one containing sunflower oil to one containing soybean oil, the fatty acid composition of the brain cells had not reached control values, e.g., that obtained in animals continuously fed soybean oil; 22:6(n-3) was 77, 65 and 80% of control levels for astrocytes, oligodendrocytes and neurons, respectively. In contrast, the recovery measured by the decay of 22:5(n-6) was complete within 10 wk. For 22:6(n-3), it took approximately 2 wk for liver and kidney to recover to the control value, 3 wk for lung, 6 wk for retina and 10 wk for testes. The decrease of 22:5(n-6) was rapid: the control values were reached within 2 wk for kidney, liver and lung and within 6 wk for retina. Because the recovery of the content of 22:6(n-3) by brain cells was very slow, the optimal ratio of long-chain fatty acid precursors must be determined very carefully. *J. Nutr.* 119: 15–22, 1989.

INDEXING KEY WORDS:

- *polyunsaturated fatty acids* • *neuron*
- *astrocyte* • *oligodendrocyte* • *brain*

The brain is a well-protected organ, with regard to polyunsaturated acids, that uses dietary fatty acids in a highly specific manner. A restriction of very short duration of the n-3 fatty acids in the diets of animals fed a complete diet causes few anomalies in the profile of polyunsaturated fatty acids in the brain and its organelles, however, in other organs the levels of these fatty acids decrease rapidly. A deficiency of n-3 fatty acids in the diet will not cause anomalies in the brain unless extremely prolonged, e.g., over several generations. In fact, a brain deficiency of polyunsaturated fatty acids will not be clearly evident unless the animals are the offspring of mothers who were already deficient during gestation.

The effects on the nerve cells of a specific deficiency in n-3 acids (with a normal supply of n-6 fatty acids) are interesting. We have previously compared animals fed a diet containing a normal amount of n-6 fatty acids but lacking in n-3 fatty acids (a diet containing sunflower oil) with animals fed a diet containing both types of fatty acids (a diet containing soybean oil) (1, 2). The brain cells and the intracellular organelles conserve a normal total quantity of polyunsaturated fatty acids, but the various cell types and organelles show a considerable deficit in cervonic acid [22:6(n-3)] that is eventually compensated for by an excess of docosapentaenoic acid [22:5(n-6)]. Comparison of animals that have been fed for 60 d either a sunflower diet or a soybean diet shows n-3/n-6 ratios of 1/20 in the diet, 1/16 in the oligodendrocytes, 1/12 in the myelin, 1/2 in the neurons, 1/6 in the synaptosomes and 1/3 in the astrocytes (1). The importance of n-3 fatty acids has also been shown by a study of phosphatidylethanolamine in animals fed a peanut or rapeseed oil diet (3, 4). A deficiency in n-3 fatty acids with a normal supply of n-6 fatty acids causes disturbances in the animals' learning capacity that parallel the anomalies in the composition of fatty acids in the cerebral phospholipids (5). This effect perhaps reflects changes in visual acuity rather than learning. This nutritional deficiency eventually causes visual disturbances and alterations in the electroretinogram (6–8). This effect also has been discussed elsewhere (9, 10).

After the transition from a sunflower diet to a soybean diet the rate of recovery is remarkably slow; it

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takes many months before the brain organelles recover a normal quantity of 22:6(n-3). This rate remains unchanged irrespective of the organelle in question (11). The recovery of myelin 22:6(n-3) composition would be expected to be somewhat slow because myelin membranes have a slow turnover rate. An unexpected observation was that the nerve endings also have a very slow rate of recovery, because the renewal of the fatty acids that form their membranes is supposed to be rapid.

The pathology of deficiency in α-linolenic acid [18:3(n-3)] has been described in the monkey (12) and in humans (13–15). A deficiency in n-3 fatty acids (16) has been suggested as a syndrome of modern societies. This work was undertaken to determine the speed of recovery of brain cells in comparison with other organs when animals previously fed a diet lacking in n-3 fatty acids (sunflower oil) were fed a diet containing n-3 fatty acids (soybean oil).

MATERIALS AND METHODS

Animals. Female Wistar rats originating from Iffa Credo (L'Arbresle, France) and bred in our laboratory were fed a purified diet containing either 1.5% sunflower oil [940 mg linoleic acid [18:2(n-6)]/100 g diet; 6 mg α-linolenic acid [18:3(n-3)]/100 g diet] or 1.9% soybean oil [940 mg 18:2(n-6)/100 g diet; 130 mg 18:3(n-3)/100 g] for three generations. The composition of the diets is shown in **Tables 1 and 2**. Because animals fed either diet ate similar amounts of food, they ate similar amounts of n-6 fatty acids. In our experimental conditions, the 18:3(n-3) deficiency had no effect on fecundity (percentage of pregnant females), fertility (number of pups/litter), pup birth weight, food intake and weight of pregnant or lactating females or pup growth during suckling. However, this deficiency did cause abnormally high rates of perinatal mortality from birth to postpartum d 3 (17). Three d after delivery, the litters were adjusted to 10 animals each. After birth, the young rats (fourth generation, male only) received the same diet as the previous three generations. From 15 d of age, half the animals fed sunflower oil were fed the diet containing soybean oil. Therefore, in these animals, the 18:3(n-3)–deficient diet was exchanged for an 18:3(n-3)–adequate diet. One half of the remaining rats fed sunflower oil were fed soybean oil from 60 d of age. Thus we determined the speed of recovery in young animals (15 d old) and in adult animals (60 d old). The oldest rat was 190 d old when it was killed. A separate group of animals was fed a nonpurified diet obtained from UAR (Villemoisson-sur-Orge, France).

Cellular and subcellular fractionation. Neurons, astrocytes (18) and oligodendrocytes (19, 20) were prepared by sieving and sucrose gradient centrifugation with minor modifications (21). Purity of the cell preparations was assessed by morphological criteria after examination of the cells with a phase-contrast microscope (19). Preparation and purity of cells have been described in our preceding papers (1, 19, 20, 22). In brief, the neuron preparation was 89% pure on the basis of morphological criteria. The remaining material con-

TABLE 1

Diet composition

Ingredient	Soybean oil	Sunflower oil
	g/kg diet	
Casein delipidated	220	220
DL-Methionine	1.6	1.6
Cellulose	20	20
Starch	459.7	463.4
Saccharose[1]	230	230
Oil	18.7	15.0
Vitamin mixture[1]	10	10
Mineral mixture[2]	40	40

[1]United States Biochemical Corp., Cleveland, OH. Composition of vitamin supplements g/kg (triturated in dextrose): α-tocopherol (1000 IU/g), 5.0; L-ascorbic acid, 45.0; choline chloride, 75.0; D-calcium pantothenate, 3.0; inositol, 5.0; menadione, 2.25; niacin, 4.5; para-amino-benzoic acid, 5.0; pyridoxine HCl, 1.0; riboflavin, 1.0; thiamin HCl, 1.0; retinyl acetate, 900,000 IU; ergocalciferol, (vitamin D-2), 100,000 IU; biotin, 20 mg; folic acid, 90 mg; vitamin B-12, 1.35 mg.

[2]Composition of the mineral mixture (per kg of diet): $CaHPO_4 \cdot 2H_2O$, 15.2; K_2HPO_4, 9.6; $CaCO_3$, 7.2; NaCl, 2.8; MgO, 0.8; $MgSO_4 \cdot 7H_2O$, 3.6; $FeSO_4 \cdot 7H_2O$, 0.28; $ZnSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot H_2O$, 0.2; $CuSO_4 \cdot 5H_2O$, 0.04; NaF, 0.04; $Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$, 0.008; KI, 0.0032; $CoCO_3$, 0.0032; $Na_2SeO_3 \cdot 5H_2O$, 0.0004.

TABLE 2

Fatty acid composition of dietary lipids[1]

Fatty acids	Diet: Sunflower oil	Diet: Soybean oil
	%	
14:0	0.3	0.3
16:0	6.4	10.1
17:0	tr[2]	0.2
18:0	3.9	5.6
20:0	0.3	0.4
22:0	0.7	0.5
Saturated	11.6	17.1
16:1	0.2	tr[2]
18:1	21.4	21.4
20:1	0.2	0.3
Monounsaturated	21.8	21.7
18:2(n-6)	66.4 (936.0)[3]	53.5 (940.0)[3]
18:3(n-3)	0.4 (6.0)[3]	7.4 (130.0)[3]
n-3/n-6	0.006	0.14

[1]Dietary fatty acid composition was analyzed by gas chromatography of methylesters under the following conditions: Packard Model 427 gas chromatograph; glass capillary column; stationary phase FFAP; gas pressure vector H_2: 0.6 bar; temperature: 190°C; detection by flame ionization.

[2]tr = Traces.

[3]Values in parentheses are levels of dietary fatty acid in mg/100 g diet.

isted of membrane fragments and some unidentified cells. Astrocytes were 85% pure (contamination was essentially due to clumps of astrocytes and membrane fragments). Oligodendrocytes were 80% pure (contamination consisted of some red blood cells; other unidentified cells possibly were small neurons and membrane fragments). As determined by radioimmunoassay, the basic protein was not detected in neurons and astrocytes, but only in oligodendrocytes. Glial fibrillary acid (GFA) protein was present only in astrocytes, as determined by rocket electrophoresis. Cells were lyophilized prior to lipid extraction.

Transmethylation and gas-liquid chromatography. Tissue and cellular lipids were extracted by sonication in chloroform/methanol 2:1 (vol/vol) (23, 24) and methylated (25). Fatty acid methylesters were separated by gas-liquid chromatography on an open tubular capillary column (0.30 mm in diameter, 45 m long) coated with free fatty acid phase (FFAP), by using a flame-ionization detector. Identification of fatty acids was performed with commercial standards by means of relative retention times. Areas were calculated with an ICAP integrator (LTT, Paris, France). Statistical methods were performed by using Student's *t*-test.

RESULTS

In 60-d-old rats fed sunflower oil, 22:6(n-3) concentration in all tissues examined, as well as in isolated brain cells, was lower and concentration in 22:5(n-6) was higher than in rats fed soybean oil (**Tables 3 and 4**). The level of 20:4(n-6) was nearly unaffected. The total amount of polyunsaturated fatty acids (n-6 and n-3) was similar in soybean- and sunflower-fed animals. Saturated and monounsaturated fatty acids were similar in both groups. Soybean-fed animals were considered to be controls because no differences were found between animals fed soybean and those fed the nonpurified diet inasmuch as the nonpurified diet contained quantities of linoleic and linolenic acid similar to those in the soybean diet. The recovery of fatty acid composition was measured by the increase in 22:6(n-3) and the decrease in 22:5(n-6) from d 60 to d 130.

Figure 1 shows that after the exchange of the sunflower diet for the soybean diet, the recovery of 22:6(n-3) was very slow for neurons, astrocytes and oligodendrocytes. Initially, the levels of 22:6(n-3) in brain cells of sunflower-fed animals were 49, 47 and 10%, respectively, of that of soybean-fed animals. Seventy d after the change in diet (130-d-old animals), the 22:6(n-3) content of these cells still had not reached the level in the soybean-fed animals. In astrocytes, the level of 22:6(n-3) was increased by approximately 75%, but it was still only 77% of that in the continuously soybean-fed rats 70 d after the change in diet. In oligodendrocytes, the increase (650%) was dramatic, but it was still only 65% of the control 70 d after the change in diet (130-d-old animals). In neurons, the increase was 180% (70% of that in the control cells).

TABLE 3

***Fatty acid profile of brain cells from 60-d-old rats fed diets containing either soybean oil or sunflower oil*[1]**

	Neurons		Astrocytes		Oligodendrocytes	
Fatty acids	Soybean	Sunflower	Soybean	Sunflower	Soybean	Sunflower
			mg/100 g			
C14:0	3.1 ± 0.2	1.8 ± 0.1	0.9 ± 0.1	2.3 ± 0.2	3.9 ± 0.3	6.5 ± 0.6
C16:0	24.3 ± 1.8	31.0 ± 1.9[a]	29.5 ± 1.2	33.0 ± 3.1	17.8 ± 1.2	20.1 ± 1.8
C16:1(n-9)	1.0 ± 0.1	0.4 ± 0.1	tr	tr	tr	tr
C16:1(n-7)	1.2 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	6.4 ± 0.9	9.4 ± 1.3
C18:0	18.9 ± 1.0	23.3 ± 1.6[a]	23.8 ± 1.5	21.0 ± 1.8	18.4 ± 1.5	16.6 ± 1.3
C18:1(n-9)	15.5 ± 1.4	15.2 ± 1.1	12.5 ± 1.4	9.7 ± 1.1	17.3 ± 1.9	16.2 ± 1.8
C18:1(n-7)	1.1 ± 0.3	2.2 ± 0.5	1.5 ± 0.2	1.0 ± 0.2	5.6 ± 0.6	4.5 ± 0.5
C18:2(n-6)	6.9 ± 0.3	2.1 ± 0.2[a]	1.2 ± 0.1	3.8 ± 0.4[a]	2.7 ± 0.2	2.9 ± 0.2
C20:0	2.4 ± 0.4	1.0 ± 0.3	0.5 ± 0.1	1.7 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
C20:1(n-9)	1.0 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	1.9 ± 0.2	1.8 ± 0.1
C20:4(n-6)	10.3 ± 1.1	7.9 ± 0.4[a]	10.3 ± 1.6	8.3 ± 0.8	9.3 ± 0.9	7.4 ± 0.6
C22:0	tr	0.8 ± 0.1	0.1 ± 0.0	1.2 ± 0.2[a]	0.3 ± 0.1	0.3 ± 0.1
C22:1(n-9)	tr	0.2 ± 0.0	tr	0.3 ± 0.0	2.4 ± 0.3	2.2 ± 0.2
C22:4(n-6)	1.0 ± 0.2	1.8 ± 0.4	2.7 ± 0.3	2.1 ± 0.2	tr	tr
C22:5(n-6)	2.2 ± 0.3	4.7 ± 0.3[a]	2.5 ± 0.2	8.6 ± 0.4[a]	3.5 ± 0.4	8.4 ± 0.6[a]
C22:5(n-3)	1.8 ± 0.4	1.5 ± 0.3	0.7 ± 0.1	tr	tr	tr
C24:0	1.0 ± 0.2	0.8 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	1.3 ± 0.2	1.4 ± 0.3
C22:6(n-3)	8.3 ± 0.4	4.1 ± 0.3[a]	12.1 ± 1.0	5.7 ± 0.3[a]	5.1 ± 0.5	0.5 ± 0.1[a]
C24:1(n-9)	tr	tr	tr	tr	1.6 ± 0.2	1.5 ± 0.2

[1]Values are means ± SEM. One group of rats was fed a sunflower oil diet through four generations. The other group was fed a soybean oil diet. Animals were killed at 60 d of age. The *n* used for statistical analysis is the number of animals; *n* = at least 48 animals, i.e., 8 litters.
[a]Significantly different from soybean oil group by at least 1% ($P < 0.01$). tr = Trace.

TABLE 4

Fatty acid profile of different organs from 60-d-old rats fed diets containing either soybean oil or sunflower oil[1]

Fatty acids	Kidney		Retina		Testicle		Lung		Liver	
	S	SF	S	SF	S	SF	S	SF	S	SF
					mg/100 g					
C14:0	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	1.7 ± 0.2	1.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
C16:0	26.1 ± 1.8	25.8 ± 1.8	22.8 ± 1.5	22.1 ± 1.6	31.2 ± 2.1	32.6 ± 2.5	39.8 ± 2.5	39.0 ± 2.4	22.5 ± 1.8	20.8 ± 1.7
C16:1(n-9)	0.6 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.8 ± 0.1[a]	0.6 ± 0.1	0.4 ± 0.1	3.0 ± 0.4	2.7 ± 0.3	0.7 ± 0.1	0.3 ± 0.1[a]
C16:1(n-7)	4.5 ± 0.3	4.1 ± 0.3	0.7 ± 0.2	1.3 ± 0.2	5.1 ± 0.5	4.2 ± 0.4	6.2 ± 0.5	5.4 ± 0.5	6.4 ± 0.6	6.2 ± 0.4
C17:0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C18:0	14.3 ± 1.0	15.4 ± 1.1	25.9 ± 2.0	26.6 ± 1.8	5.4 ± 0.4	5.6 ± 0.3	11.1 ± 1.0	12.2 ± 1.2	12.1 ± 1.9	16.1 ± 1.9
C18:1(n-9)	16.5 ± 1.9	16.0 ± 1.1	9.6 ± 0.9	10.4 ± 1.0	17.6 ± 0.9	15.0 ± 1.8	15.8 ± 1.1	15.8 ± 1.8	17.5 ± 1.2	16.0 ± 1.8
C18:1(n-7)	4.8 ± 0.3	4.0 ± 0.4	4.1 ± 0.4	4.3 ± 0.4	3.7 ± 0.2	3.1 ± 0.4	4.4 ± 0.8	3.4 ± 0.5	5.5 ± 0.8	4.0 ± 0.5
C18:2(n-6)	9.2 ± 0.5	8.2 ± 0.6	1.0 ± 0.1	0.8 ± 0.1	6.0 ± 0.8	4.7 ± 0.5	4.2 ± 0.5	4.5 ± 0.5	7.1 ± 0.9	8.1 ± 0.9
C18:3(n-6)			0.8 ± 0.1	0.2 ± 0.0[a]						
C18:3(n-3)			0.6 ± 0.1	tr	0.2 ± 0.0				0.2 ± 0.0	0.1 ± 0.0
C20:0	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	0.8 ± 0.2	0.2 ± 0.0	0.3 ± 0.0
C20:1(n-9)	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
C20:1(n-7)			0.3 ± 0.1	0.2 ± 0.0				0.4 ± 0.1		0.1 ± 0.0
C20:2(n-6)	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.1 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
C20:3(n-6)	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	1.0 ± 0.2	0.8 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	1.1 ± 0.1	1.0 ± 0.2
C20:4(n-6)	15.8 ± 1.2	17.8 ± 1.4	6.5 ± 0.4	7.8 ± 0.5	10.7 ± 1.1	12.7 ± 1.0	4.6 ± 0.5	6.9 ± 0.6	14.6 ± 1.8	17.6 ± 1.9
C20:5(n-3)	0.2 ± 0.0		0.2 ± 0.0	tr					0.4 ± 0.1	tr
C22:0	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.0			1.1 ± 0.1	1.2 ± 0.3	0.1 ± 0.0	0.3 ± 0.0
C22:1(n-9)							0.3 ± 0.1	0.2 ± 0.0		
C22:1(n-7)							0.3 ± 0.0	0.3 ± 0.0		
C22:4(n-6)	0.3 ± 0.1	0.7 ± 0.1[a]	1.2 ± 0.1	2.0 ± 0.2[a]	1.5 ± 0.1	2.0 ± 0.2	0.9 ± 0.1	1.7 ± 0.2[a]	0.4 ± 0.0	0.8 ± 0.1[a]
C22:5(n-6)	0.2 ± 0.0	0.9 ± 0.2[a]	2.7 ± 0.3	12.9 ± 0.8[a]	12.7 ± 1.0	14.3 ± 1.1	0.2 ± 0.0	0.5 ± 0.0[a]	0.7 ± 0.1	3.0 ± 0.3[a]
C22:5(n-3)			0.3 ± 0.1	tr					0.5 ± 0.1	0.1 ± 0.0
C24:0	2.3 ± 0.1	2.3 ± 0.3	1.4 ± 0.2	0.7 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	1.3 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
C22:6(n-3)	1.9 ± 0.2	0.8 ± 0.1[a]	21.8 ± 1.4	8.6 ± 0.5[a]	0.8 ± 0.0	0.3 ± 0.0[a]	0.9 ± 0.1	0.2 ± 0.0[a]	9.1 ± 0.6	3.2 ± 0.3[a]
C24:1(n-9)	1.0 ± 0.1	1.0 ± 0.1	0.3 ± 0.0	0.1 ± 0.0		0.2 ± 0.0	1.2 ± 0.2	1.1 ± 0.2	0.2 ± 0.0	0.3 ± 0.0
C24:1(n-7)							0.2 ± 0.0	0.2 ± 0.0		
C24:4(n-6)			0.1 ± 0.0	0.3 ± 0.0	1.0 ± 0.2	1.2 ± 0.2				
C24:5(n-6)			tr	0.2 ± 0.0	1.0 ± 0.1	1.2 ± 0.2				

[1]Values are mean ± SEM. One group of rats was fed a sunflower oil diet through four generations. The other group was fed a soybean oil diet. Animals were killed at 60 d of age. The *n* used for statistical analysis is the number of animals; *n* = at least 48 animals, i.e., 8 litters. [a]Significantly different from soybean oil group by at least 1% ($P < 0.01$). S = soybean oil; SF = sunflower oil. tr = Trace.

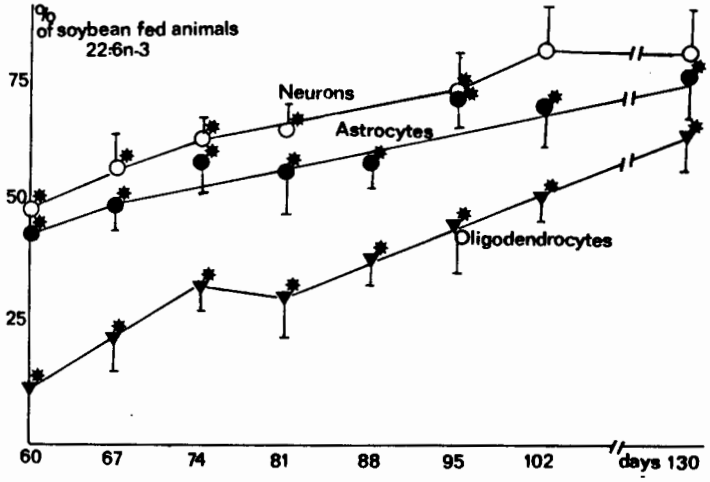

FIGURE 1 Recovery of adult brain cells as measured by 22:6(n-3) increase. Values of rats fed sunflower oil until 60 d of age, and thereafter fed soybean oil, are expressed in percentages of values obtained with animals fed soybean oil from birth. Animals were killed when 67, 74, 81, 88, 95, 102 and 130 d old (7, 14, 21, 28, 35, 42 and 70 d after changing the diet). Each point represents the mean value from at least 3 different preparations. Each preparation needed material from at least 16 animals. *Significantly different from soybean group (control) ($P < 0.01$).

In contrast, the recovery of fatty acid composition as measured by the decrease of 22:5(n-6) was complete within 70 d (**Fig. 2**). Neurons, astrocytes and oligodendrocytes from animals fed the soybean diet after 60 d of age reached the respective values found in the continuously soybean-fed animals (47, 30 and 41%, respectively, of sunflower-fed animals).

Figures 3 and 4 show that during the same period the recovery of fatty acid composition in all other organs was also complete. For liver and kidney, it took approximately 2 wk for 22:6(n-3) to reach the control value, 3 wk for lung, 6 wk for retina and 10 wk for testes.

Figure 5 shows that the recovery of 22:6(n-3) in young animals (diet exchanged at 15 d of age) was slower than in 60-d-old animals (Fig. 3): approximately 3 wk for kidney, liver and lung and 4 wk for retina. In testes, as with 22:6(n-3), the level of 22:5(n-6) was hardly affected by the change in diet. For liver and kidney, recovery time remained the same (2 wk), but it took 4 rather than 3 wk for lung. The recovery was still not complete within 6 wk for retina, and the level of 22:6(n-3) was hardly affected in testes. **Figure 6** shows that the decrease in 22:5(n-6) after the change in diet in 15-d-old

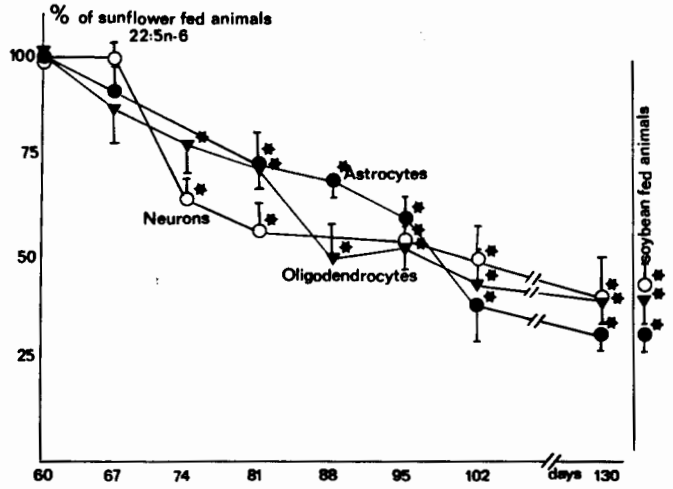

FIGURE 2 Recovery of adult brain cells as measured by 22:5(n-6) decrease. Values of rats fed sunflower oil until 60 d of age, and soybean oil thereafter, are expressed in percentages of data obtained with rats fed sunflower oil from birth. Animals were killed when 67, 74, 81, 88, 95, 102 and 130 d old (7, 14, 21, 28, 35, 42 and 70 d after changing the diet). Each point represents the mean value from at least 3 different preparations. Each preparation needed material from at least 16 animals. *Significantly different from sunflower group ($P < 0.01$).

animals was very rapid in all organs except testes. The control value was reached within 2 wk for kidney, liver and lung and within 5 wk for retina.

DISCUSSION

Since n-3 fatty acids are essential in the nervous system (26–36), it is useful to determine the minimal sup-

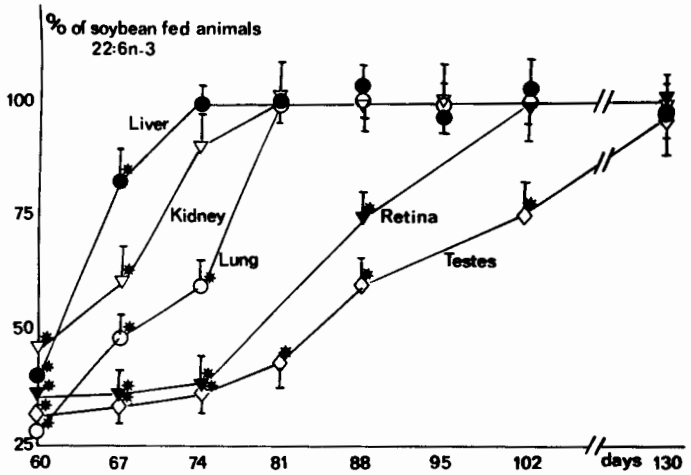

FIGURE 3 Recovery of various organs from adult animals as measured by 22:6(n-3) increase. Values of rats fed sunflower oil until 60 d of age, and soybean oil thereafter, are expressed in percentages of values obtained with rats fed soybean oil from birth. Animals were killed when 67, 74, 81, 88, 95, 102 and 130 d old (7, 14, 21, 28, 35, 42 and 70 d after changing the diet). Each point represents the mean value obtained from 14 samples (one animal per sample). The 14 animals were from at least 4 different litters. *Significantly different from soybean group ($P < 0.01$).

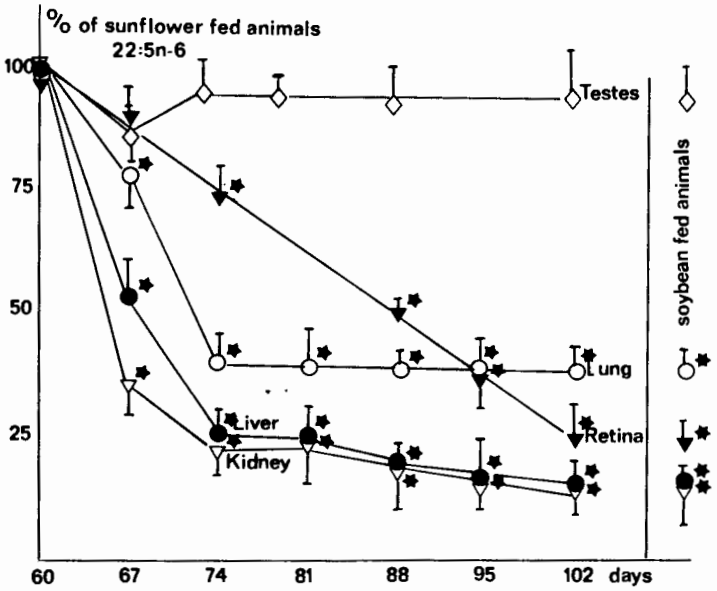

FIGURE 4 Recovery of various organs from adult animals as measured by 22:5(n-6) decrease. Values of rats fed sunflower oil until 60 d of age, and soybean oil thereafter, are expressed in percentages of values obtained with rats fed soybean oil from birth. Animals were killed when 67, 74, 81, 88, 95 and 102 d old (7, 14, 21, 28, 35 and 42 d after changing the diet). Each point represents the mean value obtained from 14 samples (one animal per sample). The 14 animals were from at least 4 different litters. *Significantly different from sunflower group ($P < 0.01$).

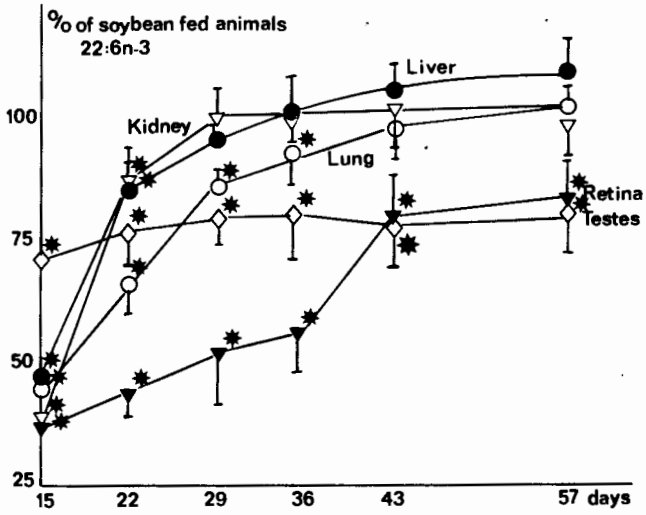

FIGURE 5 Recovery of various organs from animals as measured by 22:6(n-3) increase. Rats were fed sunflower oil until 15 d of age and soybean oil thereafter. Animals were killed when 22, 29, 36, 43 and 57 d old (7, 14, 21, 28, 42 d, respectively, after changing the diet). Values are expressed in percentages of values obtained with animals fed soybean oil from birth. Each point represents the mean value obtained from 16 samples (one analysis per animal). The 16 animals were from at least 4 different litters. *Significantly different from soybean group (control) ($P < 0.01$). The n used for statistical analysis was the number of litters (same level of significance was obtained using the number of animals).

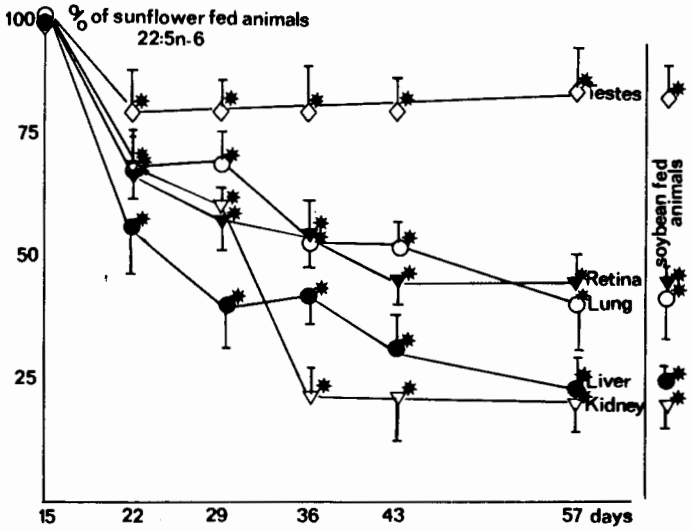

FIGURE 6 Recovery of various organs from animals as measured by 22:5(n-6) decrease. Rats were fed sunflower oil until 15 d of age and soybean oil thereafter. Animals were killed when 22, 29, 36, 43 and 57 d old (7, 14, 21, 28 and 42 d after changing the diet). Values are expressed in percentages of data obtained with sunflower-fed animals. Each point represents the mean value obtained from 16 different analyses obtained from 16 animals (one analysis per animal) from at least 4 different litters. *Significantly different from sunflower group ($P < 0.01$). The n used for statistical analysis is the number of litters (same level of significance was obtained if using the number of animals).

ply of these fatty acids which is necessary and sufficient to obtain cerebral membranes of a normal fatty acid composition. In order to achieve this, experiments involving diets which were intermediary in their 18:3(n-3) content (obtained by adding variable and increasing quantities of soybean oil to the diet) have been carried out. In all tissues examined (brain, liver, kidney, testes), the increase in the supply of 18:3(n-3) tended to increase the terminal fatty acid of the n-3 series [22:6(n-3)] and inversely to decrease the n-6 series [22:5(n-6)]. On the other hand, the increase in the hepatic level of 22:6(n-3) as dietary 18:3(n-3) increase is continuous, which clearly proves the extreme sensitivity of the liver to exogenous fatty acid supplies.

Compared with rats fed soybean oil, those fed sunflower oil showed dramatically lower 22:6(n-3) in brain, whatever the cell (neuron, astrocyte, oligodendrocyte) or organelle (myelin, mitochondria, microsomes, synaptosomes) (1). The very low amount of 18:3(n-3) in the sunflower diet was largely utilized by membranes, separated and reutilized in all organs. But the amount was not sufficient, and all organs showed a dramatic reduction in 22:6(n-3), counterbalanced by increased 22:5(n-6). The recovery of the content of 22:6(n-3) in the brain organelles (measured by feeding the sunflower-fed animals with soybean oil from either 15 d or 60 d of age) was extremely slow and required more than 8 wk (2, 11). The present work demonstrates that after the exchange of an n-6–rich diet for an n-3–rich diet, specific brain cells have a slow recovery of fatty acid composition as measured by either the increase of 22:6(n-3) or the decrease of 22:5(n-6). Moreover, we have demonstrated that under similar feeding conditions the peripheral nervous system (sciatic nerve) has a very slow recovery of 22:6(n-3) content (37).

In contrast, the recovery of the 22:6(n-3) content of liver, kidney and even lung was much more rapid (Fig. 3). Retinal recovery was slow, but this tissue is part of the central nervous system. In agreement with our present results, retina from animals fed diets in which the lipid was primarily 18:2(n-6) had decreased 22:6(n-3) and increased 22:5(n-6) (38). Interestingly, the recovery, if any, was slowest for the testes but rat testes are unique in having a high prevalence of n-6 fatty acids. In contrast, polyunsaturated fatty acids in human testes, for example, are mainly from the n-3 series (39). Our results in testes compare favorably with previously published data showing that rats fed diets containing sunflower oil have more 22:5(n-6) and less 22:6(n-3) in testes than animals fed a diet enriched with fish oil (40). Interestingly, the recovery of 22:6(n-3) composition appeared to be slower in young animals (15-d-old) than in adults (60-d-old). Although the brain is considered to be an organ heavily protected from dietary variability, a diet containing a very reduced amount of 18:3(n-3) acid affected the brain cell content of 22:6(n-3) as well as various other organs in 60-d-old animals (**Table 5**). Thus, although affected by changes in dietary lipids, as are other organs, the brain has a recovery of fatty acid composition that is appreciably slower; this could be explained by genetically programmed brain development, the lack of turnover of neurons and oligoden-

TABLE 5

***Cervonic acid [22:6(n-3)] in tissues of 60-d-old rats fed sunflower as a percentage of that in rats fed soybean oil*[1]**

Tissues	Sunflower/Soybean × 100
Neurons	49
Synaptosomes[a]	27
Oligodendrocytes	10
Myelin[a]	14
Astrocytes	47
Mitochondria[b]	25
Microsomes[b]	28
Retina	36
Sciatic nerve[c]	28
Muscle	23
Lung	27
Testes	32
Liver	35
Kidney	45

[1]For all cells, subcellular fraction and organ, the 22:6(n-3) level in rats fed sunflower oil differed from that in soybean-fed animals at $P < 0.01$. The number of animals is given in legends to tables and figures, or in the quoted references. [a]Data from ref. 1; [b]data from ref. 2; [c]data from ref. 37.

drocytes and the very slow renewal of brain membranes. Thus, when neonatal brain maturation is complete, the possibility of long-chain fatty acid synthesis is dramatically reduced and the recovery of altered fatty acid composition is very slow, if any.

For polyunsaturated fatty acids, such as 22:6(n-3), an explanation for the slow recovery could be that the rate limiting factor is the in situ synthesis of n-3 fatty acids from 18:3(n-3) due to low desaturase activities (41–43). Another explanation could be the limited transport of 18:3(n-3) or 22:6(n-3) through the blood-brain barrier (44, 45). In fact, 22:6(n-3) could be essential specifically for brain development [instead of its precursor, 18:3(n-3)], because we have previously shown that brain cells in culture preferably use 22:6(n-3) to differentiate, divide (46) and release neuromediators (47).

Previous studies have clearly demonstrated that disturbances in the profile of polyunsaturated fatty acids can alter the functioning of membrane enzymes, disturb the interactions between receptor and ligand, disrupt intercellular interactions and even upset the correct functioning of the organ (48–50). The n-3 polyunsaturated fatty acids can control certain enzymatic activities. For example, a membrane enzyme, 5′-nucleotidase, shows a very reduced activity in the brain of animals lacking in polyunsaturated fatty acids; only the addition of 18:3(n-3) restores a normal enzymatic activity (51). A diet rich in 18:3(n-3) increases n-3 fatty acid in brain capillaries and alters their prostaglandin synthesis (52).

Moreover, because changes in the degree of unsaturation in the fat of the diet are associated with alterations in both the chemical composition of lipoproteins and their metabolism by fibroblast culture (53), n-3 fatty acid deficiency could alter uptake of various molecules by brain through endothelial cells. Slow recovery in linolenic acid in nervous tissue parallels other results obtained with arachidonic acid and its low turnover (54). The optimal ratio of long-chain polyunsaturated fatty acid precursors (α-linolenic/linoleic) must be determined very carefully, especially during development.

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