Artificial Rearing of Infant Rats on Milk Formula Deficient in n-3 Essential Fatty Acids: A Rapid Method for the Production of Experimental n-3 Deficiency[1]

Glenn Ward, James Woods, Michelle Reyzer, and Norman Salem, Jr.*

Laboratory of Membrane Biochemistry and Biophysics, National Institute on Alcoholism and Alcohol Abuse, Rockville, Maryland 20852

ABSTRACT: Research into the function of docosahexaenoic acid (DHA; 22:6n-3), the predominant polyunsaturated fatty acid (PUFA) in the central nervous system (CNS), is often hindered by the difficulty in obtaining dramatic experimental decreases in DHA in the brain and retina of laboratory rats. In this study, the artificial rearing procedure, whereby infant rats are removed from their mothers, gastrostomized, and fed synthetic formula, was used in an attempt to produce rapid changes in CNS levels of DHA. Female rats were raised, from day 4–5 of life, on one of two formulas—one containing the essential fatty acids of both the n-6 and n-3 series in proportions approximately equal to those of rat milk, and the other containing high levels of 18:2n-6 but very little n-3 fatty acid. At weaning, both groups were given AIN-76A diets modified so that the PUFA content resembled that of the preweaning formula. At eight weeks of age, the n-3-deficient group exhibited decreases of more than 50% in total DHA content in the brain, accompanied by increases in arachidonic acid (AA) (20:4n-6) and, especially, docosapentaenoic acid (22:5n-6). Other artificially-reared rats were mated and their offspring were also maintained on the respective diets. In spite of the fact that they had been reared artificially, the rats mated successfully and reared litters with no obvious abnormalities. At both ten days of age and again at eight weeks, offspring of the n-3-deficient mothers exhibited decreases of more than 90% in total DHA content. Again, the long-chain n-6 PUFA increased proportionately so that total PUFA levels in the brain were not lower. As these differences are greater than those commonly reported, even after 2–3 generations of normal dietary deprivation in rodents, this procedure may be an important tool in the study of the effects of n-3 deficiency on neural development and, subsequently, of the function of DHA in nervous tissue.

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A basic principle of nutritional research is that the determination of the essentiality of a nutrient requires the manipulation of tissue levels of that nutrient and the evaluation of appropriate functional endpoints. Specifically, this strategy requires that the following criteria be met: (i) the removal of the nutrient from the food source; (ii) the maintenance of the subject for an appropriate duration so that tissue levels decline to subnormal levels; and (iii) the evaluation of changes in structure or function of that tissue. In the case of the n-3 essential fatty acids (EFA), however, this approach has proven to be difficult. Although n-3 EFA can be removed from the food source either by providing, as the sole source of fat, oils such as safflower oil or through the use of specific purified fatty acids, the second criterion (the maintenance of the subject until tissue levels of n-3 EFA have been reduced sufficiently) has been difficult to meet. The member of the n-3 family which is of major interest, docosahexaenoic acid (DHA; 22:6n-3), is the predominant polyunsaturated fatty acid (PUFA) in the central nervous system (CNS) of mammals (1–4), and DHA in the brain of many species accrues mostly from mother's milk during the suckling period, when much of the brain is undergoing the most rapid stage of development. Furthermore, DHA is selectively retained by the brain, making depletion of brain stores difficult after weaning, when brain development is almost complete and the CNS has accumulated most of the adult concentration of 22:6n-3. The depletion of DHA, if begun after weaning, can take most of the lifetime of the animal, and major decreases often require a second or even third generation to be maintained on the diet. For example, in the rat, the usual procedure has been to place postweanling females on n-3-deficient diets throughout their lives and then evaluate their offspring, and although this technique has led to drastic reductions in 22:6n-3 in the brain in the second generation according to some reports (5,6), many studies have reported more modest decreases (7–9). Continuing the dietary n-3 deprivation for a third generation resulted in further decreases (10–13), but the time and resources required to carry out such studies may be prohibitive for many researchers.

One alternative approach has been to use animals, such as the nonhuman primate or the young piglet, which can be removed from the nursing mother shortly after birth and fed a

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*To whom correspondence should be addressed at NIAAA, 12501 Washington Ave., Rockville, MD 20852.

Abbreviations: AA, arachidonic acid; AR, artificial rearing; CNS, central nervous system; DHA, docosahexaenoic acid: EFA, essential fatty acids; PUFA, polyunsaturated fatty acid.

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synthetic formula devoid of n-3 EFA throughout much of the brain development period. This method has been used successfully in a number of laboratories, producing major declines in CNS levels of DHA during early life in both monkeys (14–16) and piglets (17,18). Unfortunately, although the feeding of infant formula to monkeys and pigs provides a powerful model for the experimental production of n-3 deficiency, it has two practical limitations. Because of the considerable length of time required for these species to reach adulthood, and the space requirements for their housing and maintenance, use of these animals can require resources beyond the reach of many laboratories. Also, the common tests which would be used to evaluate functional changes in the CNS are less developed, and often more unwieldy, in these species than in common laboratory rodents.

The laboratory rat, on the other hand, is the subject of choice for most researchers. It is relatively easy and inexpensive to acquire and maintain, it reaches adulthood relatively quickly, and more is known about the behavioral capabilities of the rat than about any other nonhuman species. Furthermore, the relatively few reports of effects of n-3 deficiency suggest that DHA is required for normal retinal (12,19) and brain function (2,4,20). What is needed to facilitate further research into the nature of these effects is a method of producing rapid and dramatic declines in DHA in the rat brain.

Fortunately, there is a well-developed technique for raising infant rats on synthetic formula. The artificial rearing (AR) method for rats was developed a quarter century ago (21,22), and with subsequent advances in the development of appropriate rat-milk substitutes (23,24), it enables the researcher to make relatively precise manipulations of specific nutrients during a critical period of development (for review, see Ref. 25). AR has provided researchers with a powerful tool in such fields as early nutrition (24) and the effects of prenatal ethanol exposure (26). This paper describes the first attempt to use AR of infant rats to produce rapid decreases of DHA in the brain.

MATERIALS AND METHODS

Subjects. The first generation of rats were offspring of timed-pregnant Sprague-Dawley rats purchased from Taconic Farms (Germantown, NY). Upon arrival in our animal facility, the dams were individually housed in plastic cages on Bed o' Cobs bedding (Anderson Company, Maumee, OH) and maintained under a 12-h dark/light cycle at 21 ± 1°C. All offspring were housed, after weaning, in same-sex groups of up to five per cage.

Chow diets. Upon arrival, the pregnant dams were maintained on NIH-31 lab chow (Zeigler Brothers, Gardners, PA). After weaning, AR offspring were maintained first on modified AIN-76A powdered diets (BioServe, Frenchtown, NJ) and were eventually transferred to modified AIN-76A pelleted diets (Dyets, Inc., Bethlehem, PA). In both cases, the diet was modified in two ways: (i) the protein was comprised of vitamin-free casein, as we had determined that this protein source contained less fat than did casein; and (ii) the fat content was 5% by weight, and comprised of saturated fats in the form of distilled monoglycerides (Myverol 18-06 K; Eastman Chemicals, Inc., Kingsport, TN) and unsaturated fats in the form of ethyl esters of purified linoleate and linolenate (Nu-Chek-Prep, Elysian, MN), and docosahexaenoate (a kind gift from Professor Yasushi Tamura, Chiba University, Chiba City, Japan). The fatty acyl composition of the diets is shown in Table 1. In the control group, 12% of the fat was in the form of linoleic acid while 1% each was provided by linolenic acid and DHA. In the experimental group, there were almost no n-3 fatty acids, while 26% of the fat was in the form of linoleic acid.

Rat-milk substitute. The neonatal pups used in the AR treatment were fed a rat-milk substitute adapted from a protocol used elsewhere (J. Edmond, personal communication), and the composition is shown in Table 2. The rat-milk substitute consisted of a milk-protein substitute (provided as a gift by Professor John Edmond; UCLA, Los Angeles, CA) to which was added amino acids, carbohydrate, fat, vitamins, and minerals to approximate the composition of rat milk. During the first two days of AR, the diet was modified somewhat in that half the protein was in the form of soy protein (Supro Plus 675; Protein Technologies International, St. Louis, MO). This change was made after pilot work in our laboratory showed that partial substitution of soy protein for casein reduced mortality due to gastrointestinal bloat in the first few days of rearing. The fat content of the diet was composed of short- and medium-chain saturated fatty acids provided by medium-chain triglyceride (MCT) oil (Mead Johnson, Evansville, IN) and pure ethyl esters of the EFA linoleic and

TABLE 1
Fatty Acyl Composition of n-3-Adequate and n-3-Deficient Chow Diets[a]

	Area percent	
Fatty acid	n-3-Adequate diet	n-3-Deficient diet
Nonessential		
16:0	11.0	9.4
18:0	72.3	61.4
18:1n-9/n-12[b]	0.3	0.5
Total NEFA	85.2	72.6
n-6 PUFA		
18:2	12.0	26.3
n-3 PUFA		
18:3	1.1	0.06
22:5	0.04	ND[c]
22:6	0.8	ND
Total n-3 PUFA	2.0	0.06
Total (%)	99.2	98.9
n-3/n-6 ratio	0.163	0.002

[a]NEFA, nonessential fatty acids; PUFA, polyunsaturated fatty acids.
[b]18:1n-9 also contains n-12 isomer.
[c]ND indicates not detected.

TABLE 2
Composition of Rat-Milk Substitute

Ingredient	g/kg Diet
Protein	
Milk protein extract (SW8707)[a]	88.84
Whey protein[b]	3.21
Custom amino acid mixture[c]	0.85
Carbohydrate	
Polycose[d]	28.6
Custom fat mixture	
Custom fat mixture	123.2
Vitamins	
ICN vitamin mixture	3.52
Custom supplemental vitamin mixture[e]	0.48
Minerals	
Custom calcium mixture[f]	7.92
Noncalcium mineral mixture[g]	5.30
$CuSO_4$ solution (0.03 g/mL)	0.85
$ZnSO_4$ solution (0.38 g/mL)	0.26
Other Ingredients	
Carnitine (0.1 g/mL)	0.35
Creatine solution (0.01 g/mL)	6.16
Ethanolamine	0.03

[a]Gift from Dr. John Edmond (UCLA, Los Angeles, CA).
[b]Alacen Extra 865 (New Zealand Milk Products, Santa Rosa, CA).
[c]Arginine (512 g/kg), glycine (310 g/kg), taurine (156 g/kg), picolinic acid (22 g/kg).
[d]From Ross Laboratories (Columbus, OH).
[e]Riboflavin (16.7 g/kg), niacin (26 g/kg), pyridoxal (13.9 g/kg), inositol (929.4 g/kg).
[f]Calcium phosphate (720 g/kg), calcium chloride (205 g/kg), calcium hydroxide (75 g/kg).
[g]KH_2PO_4 (812 g/kg), $MgSO_4$ (152 g/kg), $FeSO_4$ (4 g/kg), KI (0.29 g/kg), NaF (0.246 g/kg), $AlSO_4$ (0.156 g/kg), $MnSO_4$ (0.042g/kg).

TABLE 3
Fatty Acyl Composition of n-3-Adequate and n-3-Deficient Liquid Diets[a]

	mg Fatty acid/mL diet	
Fatty acid	n-3-Adequate diet	n-3-Deficient diet
Nonessential		
8:0	48.4	36.3
10:0	22.5	18.8
16:0	0.5	0.5
18:0	0.2	0.3
18:1n-9	0.4	0.5
Total NEFA	72.5	56.9
n-6 PUFA		
18:2	12.3	38.6
n-3 PUFA		
18:3	1.2	0.03
20:5	0.01	ND[b]
22:6	0.9	ND
Total n-3 PUFA	2.2	0.03
Total (mg/mL)	87.0	95.5
n-3/n-6 Ratio	0.176	0.001

[a]Abbreviations as in Table 1.
[b]ND indicates not detected.

α-linolenic acid, and the long-chain PUFA DHA. The fatty acid composition of the two diets is shown in Table 3.

Procedure. On day 4 or 5 after birth, pups were lightly anesthetized with isofluorane and implanted with gastrostomy tubes as described elsewhere (27,28). Briefly, six-inch-long Intramedic tubing (Clay Adams, Parsippany, NJ) (PE 10, 0.61 mm o.d.) was lubricated with MCT oil, inserted into the mouth, passed down the esophagus and out the lateral wall of the stomach. The pups were then housed on bedding in small plastic containers floating in heated water (36 ± 1°C). The milk substitute was delivered for 10 min every hour *via* PE 50 tubes attached to syringes mounted on infusion pumps (Harvard Apparatus, South Natick, MA). The total amount of food delivered each day was equal to 33% of the body weight of the pup for the first 3–4 d, and then gradually increased over the next week until it was 40% of body weight each day. Approximately equal numbers of pups from each litter were assigned to each experimental group. Pups were weaned on days 18–19 onto solid diets corresponding in PUFA content to the liquid diets. Some AR rats were killed at approximately eight weeks of age for lipid analysis, and others were allowed to reach sexual maturity and were mated beginning at 16 wk of age. Their offspring were allowed to suckle with their mothers and were removed and housed in same-sex groups on day 25 ± 1 d. They were then fed the diets fed to their mothers. Some pups were killed by decapitation on day 10 for fatty acid analysis, and others were killed by CO_2 exposure at eight weeks of age. In both cases, no more than one animal from each litter was used for biochemical analysis. All animal procedures were carried out in accordance with the guidelines set by the Animal Welfare Act and were done with the approval of the NIAAA Animal Care and Use Committee.

Biochemical analysis. Lipids were extracted from brain homogenates with chloroform and methanol by the method of Bligh and Dyer (29). Fatty acid methyl esters of total lipids were prepared with 14% (wt/vol) boron trifluoride and methanol according to the procedure of Morrison and Smith (30), and analyzed by a gas chromatograph (Hewlett-Packard 5890; Hewlett-Packard, Palo Alto, CA) equipped with a flame-ionization detector and a 30 m × 0.25 mm (i.d.) capillary column (J&W DB-FFAP; J&W, Folsom, CA). The conditions were as follows: 130°C initial temperature, no hold time, increase 4°C/min to 175°C, 1°C/min to 210°C, 30°C/min to 240°C, hold for about 15 min. Individual fatty acids were quantified by adding known amounts of the fatty acid 23:0 to the original homogenate before extraction, and the fatty acids were identified by comparison with known standards.

Statistical analysis. Data were analyzed as μg of fatty acid per gram of tissue by *t*-tests using the StatView statistical analysis package for the Macintosh computer. Significance level was set at $P < 0.01$.

RESULTS

The forebrain and cerebellar fatty acid composition of adult rats reared artificially from days 4–5 of life are shown in

TABLE 4
Effects of Feeding n-3-Deficient Diet Beginning on Day Five of Life in Adult (8 wk) Rat Forebrain Fatty Acyl Composition[a]

	μg/g Forebrain (wet weight)		
Fatty acid	n-3-Adequate diet	n-3-Deficient diet	*P*-value
Nonessential			
14:0	163 ± 3	107 ± 8	0.0245
16:0	4611 ± 27	4931 ± 42	0.0217
16:1n-7	102 ± 2	103 ± 1	0.8316
18:0	4546 ± 25	4889 ± 34	0.0070
18:1n-9	3618 ± 15	3699 ± 36	0.3789
18:1n7	841 ± 7	930 ± 10	0.0110
Total NEFA	15174 ± 75	16046 ± 136	0.0365
n-6 PUFA			
18:2	81 ± 1	86 ± 1	0.2027
20:3	106 ± 2	82 ± 2	0.0022
20:4	2267 ± 15	2642 ± 11	<0.0001
22:4	680 ± 3	917 ± 6	<0.0001
22:5	150 ± 3	1964 ± 34	<0.0001
Total n-6 PUFA	3301 ± 20	5715 ± 42	<0.0001
n-3 PUFA			
22:5	36 ± 1	9 ± 0	<0.0001
22:6	3259 ± 22	1666 ± 21	<0.0001
Total n-3 PUFA	3298 ± 21	1674 ± 21	<0.0001
Total (ug/g)	21773 ± 112	23436 ± 181	
n-3/n-6 Ratio	1.00	0.29	<0.0001

[a]Data are expressed as the mean ± SEM for n = 5 animals each. Abbreviations as in Table 1.

TABLE 5
Effects of Feeding n-3-Deficient Diet Beginning on Day Five of Life in Adult (8 wk) Rat Cerebellum Fatty Acyl Composition[a]

	μg/g Cerebellum (wet weight)		
Fatty acid	n-3-Adequate diet	n-3-Deficient diet	*P*-value
Nonessential			
14:0	180 ± 17	231 ± 9	0.2796
16:0	4148 ± 78	4945 ± 128	0.0449
16:1n-7	177 ± 17	219 ± 9	0.3627
18:0	4509 ± 73	4892 ± 169	0.3796
18:1n-9	4190 ± 69	4267 ± 185	0.8656
18:1n-7	1192 ± 24	1343 ± 51	0.2628
Total NEFA	16780 ± 289	18608 ± 708	0.3163
n-6 PUFA			
18:2	131 ± 3	137 ± 5	0.6770
20:3	129 ± 3	122 ± 5	0.5682
20:4	1810 ± 34	2292 ± 86	0.0474
22:4	567 ± 13	993 ± 39	0.0016
22:5	102 ± 3	2408 ± 85	<0.0001
Total n-6 PUFA	2786 ± 55	6015 ± 208	0.0002
n-3 PUFA			
22:5	49 ± 1	12 ± 1	<0.0001
22:6	3687 ± 63	1550 ± 41	<0.0001
Total n-3 PUFA	3736 ± 64	1563 ± 41	<0.0001
Total (ug/g)	23302 ± 407	26185 ± 945	
n-3/n-6 ratio	1.34	0.26	<0.0001

[a]Data are expressed as the mean ± SEM for n = 5 animals each. Abbreviations as in Table 1.

Tables 4 and 5. At eight weeks of age, the levels of n-3 PUFA, particularly 22:6n-3, in both the forebrain and cerebellum declined considerably in the group reared on n-3-deficient rat-milk substitute neonatally and weaned onto deficient chow, and this decrease was accompanied by an increase in the amount of n-6 PUFA, particularly 22:5n-6 and, to a lesser extent, 20:4n-6 and 22:4n-6. The percentage decline of 22:6n-3 in the cerebellum was slightly greater than that in the forebrain (58% vs. 49%).

Most AR rats mated successfully and gave birth to live litters (15/16 in the adequate group vs. 12/15 in the deficient group). In several cases in both groups, all pups within a litter died within a few days of birth (five litters in the adequate group vs. three litters in the deficient group). Under visual examination, surviving pups appeared to be normal in both groups. Because the precise day of copulation was not known in these animals, it is not known if sexual responsiveness or gestation length differed between groups.

The fatty acid composition of brain regions of ten-day-old offspring of AR rats is shown in Tables 6 and 7. The n-3 PUFA declined by over 90% in both the forebrain and cerebellum of the n-3-deficient pups, and again, this drop was accompanied by increases in n-6 fatty acids. Similar declines of 22:6n-3 and associated increases in 22:5n-6 and other n-3 PUFA were also seen in the forebrain and cerebellum of eight-week-old offspring (Tables 8 and 9).

DISCUSSION

In this study, rats which had been fed n-3-deficient diets from day 4–5 of life and throughout development and adulthood exhibited losses of approximately 50% in n-3 fatty acids—particularly 22:6n-3—in the brain. Furthermore, their offspring exhibited losses of greater than 90% in 22:6n-3 in the brain both during the neonatal period and in early adulthood. These losses were offset by increases in long-chain n-6 fatty acids—primarily 22:5n-6, but also 20:4n-6 and 22:4n-6—so that the total PUFA content of the brain was maintained as described previously (31,32).

Although a recent study reported dramatic decreases in 22:6n-3 in the brains of offspring of guinea pigs fed n-3-deficient diets from weaning (33), the decreases found in the current study are of a much greater magnitude than those reported in rats or mice in most studies which rely on feeding of n-3-deficient laboratory chow beginning at weaning in the first generation. Although some early reports (5,6) described reductions of as much as 90–95% in phospholipid 22:6n-3 in the brain in the second generation, later studies generally have reported more modest decreases of approximately 50–75% (7–9). Even when the dietary n-3 deprivation was continued for a third generation, the resulting 22:6n-3 levels were still between one-half to one-quarter of those of n-3-adequate controls (10–13),

TABLE 6
Fatty Acyl Composition of Forebrain in Ten-Day-Old Rat Pups of Artificially-Reared Dams[a]

	μg/g Forebrain (wet weight)		
Fatty acid	n-3-Adequate diet	n-3-Deficient diet	*P*-value
Nonessential			
14:0	201 ± 5	214 ± 4	0.3034
16:0	3675 ± 55	3878 ± 92	0.3829
16:1n-7	168 ± 3	177 ± 3	0.3780
18:0	2085 ± 26	2151 ± 58	0.6212
18:1n-9	1354 ± 14	1330 ± 32	0.7435
18:1n-7	371 ± 4	380 ± 9	0.6357
Total NEFA	7918 ± 103	8202 ± 197	0.5470
n-6 PUFA			
18:2	105 ± 3	98 ± 1	0.2781
18:3	5 ± 0	6 ± 0	0.2708
20:3	79 ± 3	48 ± 0	0.0012
20:4	1719 ± 20	2044 ± 50	0.0237
22:4	337 ± 4	484 ± 10	0.0004
22:5	232 ± 11	1712 ± 51	<0.0001
Total n-6 PUFA	2495 ± 38	4406 ± 108	0.0002
n-3 PUFA			
22:5	27 ± 0	3 ± 0	<0.0001
22:6	1766 ± 22	130 ± 4	<0.0001
Total n-3 PUFA	1799 ± 22	134 ± 4	<0.0001
Total (ug/g)	12212 ± 161	12742 ± 309	
n-3/n-6 Ratio	0.72	0.03	<0.0001

[a]Data are expressed as the mean ± SEM for n = 4 animals each. Abbreviations as in Table 1.

TABLE 7
Fatty Acyl Composition of Cerebellum in Ten-Day-Old Rat Pups of Artificially-Reared Dams[a]

	μg/g Cerebellum (wet weight)		
Fatty acid	n-3-Adequate diet	n-3-Deficient diet	*P*-value
Nonessential			
14:0	179 ± 12	145 ± 13	0.3527
16:0	2102 ± 141	1720 ± 136	0.3696
16:1n-7	107 ± 8	87 ± 6	0.3715
18:0	1232 ± 80	1041 ± 84	0.4353
18:1n-9	1102 ± 91	892 ± 72	0.4177
18:1n-7	305 ± 24	253 ± 19	0.4512
Total NEFA	5156 ± 359	4249 ± 334	0.3963
n-6 PUFA			
18:2	80 ± 6	61 ± 5	0.3203
18:3	3 ± 0.2	2 ± 0.2	0.5031
20:3	51 ± 4	28 ± 2	0.0665
20:4	922 ± 62	894 ± 76	0.8843
22:4	227 ± 15	286 ± 27	0.3148
22:5	121 ± 7	684 ± 59	0.0013
Total n-6 PUFA	1415 ± 92	1965 ± 169	0.1546
n-3 PUFA			
22:5	23 ± 2	2 ± 0.4	0.0087
22:6	975 ± 61	80 ± 8	0.0016
Total n-3 PUFA	1002 ± 63	82 ± 9	0.0017
Total (ug/g)	7573 ± 514	6296 ± 511	
n-3/n-6 Ratio	0.710	0.041	<0.0001

[a]Data are expressed as the mean ± SEM for n = 4 and n = 3 animals each, respectively. Abbreviations as in Table 1.

considerably higher than those of the second generation in this study.

When rats were given n-3-deficient diets beginning during the neonatal period, the losses of 22:6n-3 in the cerebellum exceeded those in the forebrain, and both structures exhibited similar losses when deficiency occurred throughout all stages of development. This may have been due to the fact that, in the rat, the cerebellum undergoes most of its development—and subsequent accrual of n-3 fatty acids—during the postnatal period while the forebrain has already undergone much of its development at the time of birth (34).

It has been reported in a number of studies (10,35,36) that n-3 deficiency is associated with increased frequency of reproductive failure, and n-3 fatty acids are reported to be necessary for the normal uterine development in the female rat (37). Some of the AR rats in the present study exhibited some difficulty in caring for their pups in the first few days after birth, but the frequency of this finding was approximately similar in both deficient and adequate dams. It is more likely that this problem was caused by the early removal of the rats from their own mothers and/or the AR experience itself. Future studies will address this issue in a quantitative fashion by evaluating maternal behavior in deficient and in adequate dams.

Earlier, the point was made that the determination of the essential nature of a nutrient required the removal of the nutrient from the food source, the maintenance of the subject for an appropriate duration so that tissue levels decline to subnormal levels, and the evaluation of any change in structure or function of that tissue. Because much of the DHA in the brain has accrued by weaning in most mammalian species, dietary n-3 deprivation beginning after this period may be relatively inefficient due to the time required to substantially reduce DHA levels in the brain. In species such as the primate or pig, feeding of n-3-deficient formulae can eliminate dietary n-3 EFA during brain development, but practical considerations make the use of these species difficult for many researchers. The rat, on the other hand, is an ideal experimental subject due to its relatively brief generation time, the relative ease with which it can be housed and maintained, and the vast amount of information available regarding its behavioral repertoire. In this study, through the use of the AR method, we have shown that the rat may also be an ideal subject for studies requiring dietary deprivation during much of the period of brain development. Future work in our laboratory will further address the precise nature of these biochemical changes at the level of the individual phospholipid classes and molecular species, and the question of functional changes in

TABLE 8
Fatty Acyl Composition of Forebrain in Adult Offspring of Artificially-Reared Dams[a]

	μg/g Forebrain (wet weight)		
Fatty acid	n-3-Adequate diet	n-3-Deficient diet	*P*-value
Nonessential			
14:0	216 ± 7	206 ± 3	0.5972
16:0	5366 ± 50	5152 ± 28	0.1364
16:1n-7	163 ± 5	151 ± 2	0.3433
18:0	5043 ± 41	4787 ± 33	0.6612
18:1n-9	4129 ± 48	3622 ± 15	0.0019
18:1n-7	930 ± 10	920 ± 5	0.6957
Total NEFA	17179 ± 174	16196 ± 59	0.0435
n-6 PUFA			
18:2	75 ± 1	75 ± 1	0.9887
20:3	104 ± 3	73 ± 1	0.0010
20:4	2553 ± 13	2728 ± 13	0.0028
22:4	771 ± 6	1011 ± 5	<0.0001
22:5	208 ± 5	3209 ± 13	<0.0001
Total n-6 PUFA	3726 ± 17	7114 ± 30	<0.0001
n-3 PUFA			
22:5	29 ± 1	ND[b]	<0.0001
22:6	3734 ± 36	307 ± 5	<0.0001
Total n-3 PUFA	3763 ± 36	307 ± 5	<0.0001
Total (ug/g)	24668 ± 222	23617 ± 84	
n-3/n-6 Ratio	1.01	0.04	<0.0001

[a]Data are expressed as the mean ± SEM for n = 4 animals each. Abbreviations as in Table 1.
[b]ND indicates not detected.

TABLE 9
Fatty Acyl Composition of Cerebellum in Adult Offspring of Artificially-Reared Dams[a]

	μg/g Cerebellum (wet weight)		
Fatty acid	n-3-Adequate diet	n-3-Deficient diet	*P*-value
Nonessential			
14:0	130 ± 13	147 ± 3	0.5867
16:0	3941 ± 100	4224 ± 69	0.3270
16:1n-7	128 ± 13	135 ± 3	0.8265
18:0	3989 ± 72	3941 ± 62	0.8272
18:1n-9	3722 ± 92	3369 ± 58	0.1848
18:1n-7	1002 ± 23	1044 ± 15	0.5223
Total NEFA	14644 ± 336	14679 ± 234	0.9698
n-6 PUFA			
18:2	97 ± 2	104 ± 2	0.2736
20:3	100 ± 3	87 ± 1	0.1204
20:4	1636 ± 19	1968 ± 35	0.0058
22:4	498 ± 6	905 ± 16	<0.0001
22:5	115 ± 3	2785 ± 42	<0.0001
Total n-6 PUFA	2474 ± 29	5889 ± 95	<0.0001
n-3 PUFA			
22:5	30 ± 1	6 ± 0.3	<0.0001
22:6	3317 ± 67	314 ± 2	<0.0001
Total n-3 PUFA	3347 ± 67	320 ± 2	<0.0001
Total (ug/g)	20465 ± 420	20889 ± 327	
n-3/n-6 Ratio	1.35	0.06	<0.0001

[a]Data are expressed as the mean ± SEM for n = 4 animals each. Abbreviations as in Table 1.

the CNS resulting from severe DHA deficiency in neural tissue.

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