Physical and Neurological Development of the Progeny of Female Rats Fed an Essential Fatty Acid-Deficient Diet During Pregnancy and/or Lactation[1]

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ABSTRACT Female Wistar rats, fed ad libitum a purified diet containing 10% corn oil for approximately 2 weeks prior to mating and during the first 14 days of gestation, were fed the same diet or transferred to one containing hydrogenated coconut oil (essential fatty acid (EFA)-deficient) in place of the corn oil, for the last 7 days of gestation. At parturition, females from each group were fed either the same diet or the alternate diet. Physical, neuromotor and reflexologic development of the progeny was determined during lactation. Omission of dietary essential fatty acids from the diet of the dam during the last week of gestation did not adversely affect litter size, total litter weight or mean birth weight of the progeny. The physical development of the pups during lactation was not significantly affected by the nature of the dietary fat consumed by the dam during gestation and lactation. Maternal dietary fat did influence the acquisition by the progeny of such reflexes as audicular startle, cliff drop aversion, negative geotaxis and bar holding. This was evident if the EFA deficiency was imposed only during the final week of gestation but was accentuated by extension of the deficiency into lactation. Onset of neuromotor activities such as head waving, crawling and standing was not delayed by lack of EFA in the maternal diet. Omission of EFA from the maternal diet during gestation did not reduce the total time spent in neuromotor activity by the progeny, whereas omission of EFA from the maternal diet during lactation did reduce the time spent standing by the progeny. Lack of polyunsaturated fatty acids from the maternal diet during the final week of pregnancy and/or throughout lactation appeared to be less detrimental to the physical, reflexological and neuromotor development of the progeny than did lack of energy, protein or pyridoxine, nutrients previously investigated by other workers. J. Nutr. *108:* 351–357, 1978.

INDEXING KEY WORDS maternal dietary fat · essential fatty acids · physical development · reflexologic development · neuromotor co-ordination

In recent years, it has become very apparent that a variety of nutritional deficiencies, imposed during pregnancy and/or lactation, will adversely affect the outcome of pregnancy and the subsequent development of the progeny. The consequences of such deficiencies range from slightly impaired growth through physical and neurologically related performance deficits to overt physical malformities, still-births and resorption of the fetus (1–10).

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It has long been known that lack of dietary essential fatty acids (EFA)[3] during pregnancy can result in prolonged gestation, excessive hemorrhaging at parturition and outright reproductive failure; live progeny born to deficient females seldom survive to weaning (1). Successful parturition and survival of the progeny are possible if linoleic acid is withdrawn from the diet only during the latter stages of pregnancy (2) or if the diet fed to the dam throughout pregnancy is only moderately deficient in essential fatty acids (3). By employing such techniques, Paoletti & Galli (2) were able to demonstrate the long term effects of maternal EFA deficiency on the avoidance behavior of the offspring. The objectives of the present study are to determine the effects of limited restriction of polyunsaturated fatty acids in the diet of the dam during pregnancy and/or lactation on the physical, reflexologic and neuromotor development of the progeny, and to ascertain the extent to which any adverse effects are corrected by nutritional rehabilitation at birth or weaning.

MATERIALS AND METHODS

Virgin female Wistar rats[4] weighing 150 to 200 g, which had been fed a closed formula, cereal based stock diet from weaning, were housed individually in galvanized steel cages and fed ad libitum a purified diet for approximately 2 weeks prior to mating. The diet contained, by weight, dextrose, 59%; casein, vitamin-free, 20%; corn oil, 10%; cellulose, 6.5%; salt-mix, Williams-Briggs modified formulation (12), 3.5%; and vitamin mix, 1% (13). The corn oil contained the following fatty acids (by weight): 16:0, 10.3%; 18:0, 1.8%; 18:1, 24.9%; 18:2, 61.4%; 18:3, 1.1%; minor components, 0.5%. In the hydrogenated coconut oil, the major fatty acids were (by weight): 8:0, 6.6%; 10:0, 7.2%; 12:0, 52.6%; 14:0, 17.1%; 16:0, 7.5%; 18:0, 8.6%, 18:1, 0.3%; 18:2, 0.1%. Vaginal smears were examined to determine the stage of estrus. Females in estrus were mated with individual male rats; sperm-positive females were considered to be pregnant after mating.

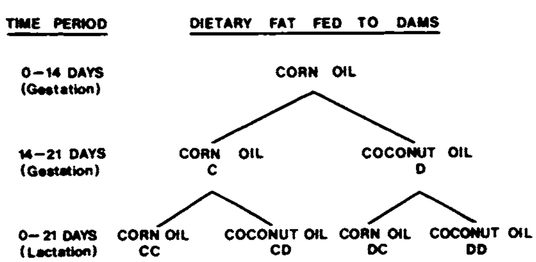

Fig. 1 Design of dietary treatments of female rats during pregnancy and lactation. C and D denote the corn oil and hydrogenated coconut oil diets, respectively, and the first and second letters indicate the dietary fat of the dam during gestation and lactation, respectively.

Fourteen days after mating, the rats were divided into two groups and the larger group was fed an EFA-deficient diet in which corn oil was replaced by hydrogenated coconut oil. Throughout gestation and lactation the food intakes and weight gains of the dams were monitored. At parturition the groups of females and their offspring were subdivided according to the scheme presented in figure 1.

At parturition, litter size, total litter weight and mean birth weight of the pups were recorded. The litters were culled to eight pups by retaining the median animals by weight and were color coded. Physical development of the pups during lactation was assessed by recording the day of onset and completion, within each dietary group, of the unfolding of the external ear, eruption of incisors, opening of eyes and growth of anogenital hair. Body weight was measured at weaning. Crawling, head waving, standing supported and standing unsupported were employed as indicators of neuromotor development. The average day of onset of each activity was recorded for each group of progeny. The time spent in these activities was also recorded. The times spent in grooming and total relative activity during a fixed test-period were assessed as parameters of spontaneous activity. The equipment and detailed procedures employed in making these observations are described elsewhere (3).

The pups were also subjected to a battery of reflexologic tests during lactation.

[3] Abbreviations: EFA, essential fatty acid(s); X:YωZ, fatty acid with X carbon atoms and Y double bonds with the terminal double bond Z carbon atoms from the methyl group.
[4] Woodlyn Farms, Guelph, Ontario.

The righting reflex was recorded as positive if the animal turned over to rest in normal position within 2.5 seconds of being placed on its back on a hard flat surface. If a loud sharp buzzer elicited sudden extension of head and limbs, the audicular startle test was recorded as positive. Onset and completion of cliff avoidance (pup turned and crawled away when placed with its forepaws over the edge of the bench), palmar grasp (digits flexed to grasp a paper clip used to stroke forepaw), negative geotaxis (pup turned and crawled uphill when placed on a 20° slope), vibrissa placing (pup raised head and extended forelimbs to grasp bench when lowered by tail so that vibrissa contacted bench), and visual placing (pup attempted to grasp bench when lowered by tail without touching bench) were also determined. In each instance the days of onset and completion of positive responses were recorded for each dietary group. Detailed descriptions of these responses have been published elsewhere (9, 14, 15).

Data were subjected to analysis of variance and Duncan's multiple range test (16) for statistical assessment.

RESULTS

One of the initial 27 female rats was found to be infertile and was removed from the experiment and replaced by a fertile female. In the group fed corn oil, one dam and her pups died before parturition and all of the pups of a second dam died at birth. A long gestational period of 26 days, with subsequent death of all the pups, was recorded for one dam in the group fed the EFA-deficient diet during the final week of pregnancy. The above animals are not included in the data presented. Six dams remained in each of the four dietary groups (CC, CD, DC and DD) during lactation.

The total food intake during the final week of pregnancy was significantly lower for dams fed coconut oil than for dams fed corn oil (164.5 versus 174.5 g; $S\bar{x} = 5.5$); a significantly lower weight gain per week was also observed (85.4 versus 96.8 g; $S\bar{x} = 5.7$). However, the weekly food intakes per pup (12.4 and 12.4 g; $S\bar{x} = 0.2$) and weight gains per pup (6.8 and 6.5 g; $S\bar{x} = 0.2$) were similar for dams fed corn oil and coconut oil respectively. During lactation, the food intakes per pup and weight changes of the dams per pup did not differ significantly among the four groups of rats.

The nature of the dietary fat during the final week of gestation did not significantly affect the mean litter size (14.4 and 13.7) or mean number of live pups per litter (14.2 and 13.3) born to dams fed corn oil and coconut oil respectively. Data represent means for 12 dams per group. The smallest litter size in the corn oil group was seven and in the deficient group it was eight. The largest litter of 16 pups was born to a dam fed coconut oil. The total litter birth weights and mean birth weights of the pups were not adversely affected by feeding the deficient diet during gestation, nor were the weight gains of the pups significantly affected by the nature of the dietary fat fed to the dam during gestation and lactation (39.8, 39.0, 39.5 and 38.6 g for groups CC, CD, DC and DD respectively).

Pups in all four dietary groups developed physically at the same rate during lactation. Onset and completion of unfolding of the outer ear flap (days 3 and 4 respectively), eruption of incisors (days 10 and 12), opening of eyes (days 14 and 16) and growth of anogenital hair (days 15 and 19), were identical in all four dietary groups. The average age of onset of the neuromotor skills, head-waving (2.0 days), crawling (4.5 days), standing supported (12.0 days), and standing unsupported (16.0 days), were identical for groups CC, CD and DC. Pups in group DD began head-waving and crawling at the same age as those in the other three groups but were slightly older when they first stood supported (13.2 days) or unsupported (16.5 days). The differences were not significant.

Results of reflex testing of rat pups are shown in table 1. Righting reflex, cliff drop aversion, and negative geotaxis appeared early in the first week of lactation, whereas bar-holding ability, startle reflex to sound, visual placing and vibrissa placing appeared in the second week of lactation. There were no differences in the onset and completion of the righting reflex on a flat surface, visual placing and vibrissa placing,

TABLE 1

Effect of maternal dietary fat on the onset and completion of reflex acquisition in rat pups

Reflex	CC[1] Day[2] Onset	CC[1] Day[2] Completion	CD Day Onset	CD Day Completion	DC Day Onset	DC Day Completion	DD Day Onset	DD Day Completion
Righting reflex	3	6	3	6	3	6	3	6
Cliff drop aversion	3	6	3	6	3	8	4	8
Negative geotaxis	3	7	3	8	3	8	4	8
Bar-holding ability	9	12	9	13	9	13	10	14
Startle reflex	12	14	12	15	13	16	14	16
Vibrissae placing	12	13	12	13	12	13	12	13
Visual placing	14	16	14	16	14	16	14	16

Dietary group: CC, CD, DC, DD.

[1] C and D denote corn oil and hydrogenated coconut oil respectively; the first and second letters indicate the dietary fat of the dam during gestation and lactation respectively. There were six litters of eight pups in each dietary group. [2] The day of onset was the day on which the reflex was first acquired by any pup in the dietary group; the day of completion was the day on which all pups in the group had acquired the reflex.

among the various groups. In comparison with the control rats, the day of onset of startle reflex to sound was delayed in groups DD and DC and was completed later in groups CD, DC and DD. Cliff drop aversion was first acquired and completed later in group DD, it was also completed later in group DC. Group CD did not differ from the control group. Onset of negative geotaxis and bar-holding ability was delayed and completed later in group DD; in groups CD and DC the completion of acquisition of these reflexes was delayed in comparison with control rats.

The average total times pups spent standing supported and unsupported during 6 minute observational periods are presented in table 2. Groups receiving the control diet during lactation (CC and DC) did not differ significantly on either of the test days. Pups suckling dams fed the deficient diet during lactation spent less time in these activities. There were no consistent differences in the number of head-lifting reactions attempted by pups in the four dietary groups. Grooming activity was significantly lower in pups suckling dams fed coconut oil during lactation (CD and DD). This became apparent in group DD by day 16 of lactation and was also observed in group CD by day 21 (table 3).

The average total relative activity of the

TABLE 2

Effect of maternal dietary fat on neuromotor co-ordination (standing) of rat pups

Dietary group	Time (secs) spent standing[1,2] Supported Day 16	Supported Day 21	Unsupported Day 16	Unsupported Day 21
CC	13.6^{a}	25.3^{a}	12.1^{a}	19.6^{a}
CD	10.1^{b}	20.2^{ab}	9.6^{ab}	11.9^{b}
DC	14.6^{a}	27.4^{a}	11.4^{a}	22.4^{a}
DD	9.9^{b}	20.1^{b}	7.6^{b}	12.1^{b}
SEM	1.0	1.9	1.0	2.7

[1] Time in seconds during a six minute observation period. [2] Each value represents the mean of 48 pups. Treatment means not sharing a common superscript letter are significantly different ($P < 0.05$) by Duncan's Multiple Range Test, SEM is the overall standard error of the mean.

TABLE 3

Effect of maternal dietary fat on grooming activity of rat pups

Group	Time (seconds) spent in grooming[1,2] Day 16	Day 21
CC	43.8^{a}	101.5^{a}
CD	44.9^{a}	84.3^{b}
DC	42.1^{a}	107.1^{a}
DD	31.7^{b}	90.5^{b}
SEM	3.0	5.2

[1] Time in seconds during a 6 minute observation period. [2] Each value represents the means of 48 pups. Means not sharing a common superscript letter are significantly different ($P < 0.05$) by Duncan's Multiple Range Test. SEM is the overall standard error of the mean.

pups in the various groups is shown in table 4. On days 3, 6 and 8 the relative activities of the experimental rats did not differ significantly from the control rats and are omitted from the table. On day 10, the activity of pups from groups CC and DD was lower than that of pups from dams whose diets were different during gestation and lactation (CD and DC). On days 16 and 21, pups from group DC were more active than those in the other three groups.

TABLE 4

Effect of maternal dietary fat on total relative activity of rat pups

Group	Total relative activity (pulses)[1,2]		
	Day 10	Day 16	Day 21
CC	307.7[a]	564.7[a]	789.6[a]
CD	420.2[b]	552.7[a]	756.3[a]
DC	400.4[a]	612.6[a]	860.9[a b]
DD	300.9[b]	520.4[b]	671.3[b]
SEM	30.89	22.1	41.25

[1] Six minute observation periods. Numbers represent impulses recorded on chart. [2] Each value represents the mean of 48 pups. Means not sharing a common superscript letter are significantly different ($P < 0.05$) by Duncan's Multiple Range Test. SEM is the overall standard error of the mean.

DISCUSSION

In this study, EFA deficiency was only imposed on the dams after 2 weeks of gestation. This design was adopted after a preliminary investigation revealed that introduction of the deficient diet at that time resulted in survival rates of the progeny comparable to those observed with dams fed the control diet throughout gestation. Imposition of the deficiency at mating or after 1 week of gestation resulted in high mortality among the offspring.

Feeding an EFA-deficient diet to female rats during the final week of gestation and/or lactation did not impair growth or the physical development of the progeny in this study. Paoletti and Galli (2) reported slightly lower body weights in 10 day old pups born to dams fed a fat-free diet, rather than one containing 2% corn oil, during the final week of pregnancy. The differences in total dietary fat between the earlier study (2) and the current one, and indeed, the slight difference in the fat contents of the diets used in the earlier study, render direct comparisons difficult, although both investigations involved EFA deficiency. If the maternal diet during pregnancy and/or lactation was deficient in energy (4–6), protein (6, 17) or pyridoxine (8, 9, 18), the growth of the progeny prior to weaning was impaired. Eye-opening and growth of anogenital hair were also impaired in progeny of dams fed B_6-deficient diets during lactation (18). Obviously, omission of essential fatty acids from the maternal diet during pregnancy and/or lactation was less detrimental to the physical development of the progeny than lack of energy, protein or pyridoxine.

Since the EFA-deficient diet was only introduced during the final week of pregnancy, it is conceivable that the dam mobilized stores of EFA during lactation (cf. Galli (19)) and that the supply of these acids in the milk, though lower than that in control dams, was adequate to sustain normal growth in the progeny throughout lactation. That essential fatty acids were available to pups nursing dams fed the deficient diet became evident in a subsequent study in which the ω6 fatty acid content of the brain lipids was found to increase between birth and weaning (20).

Delayed acquisition of reflexes has been observed in the progeny of pyridoxine-restricted dams (9) and of dams with a restricted energy intake (21) during gestation. Slight delays in reflexologic development were noted in progeny of dams fed the EFA-deficient diet only during gestation (group DC). In all instances, the startle reaction to sound was most severely affected. Maternal EFA deficiency appeared to be less detrimental than lack of energy (21) or pyridoxine (9) in this respect. Feeding the EFA-deficient diet during both gestation and lactation (group DD) accentuated the problem, a situation also observed by Simonson et al. (21) in progeny of underfed females. However, progeny of dams fed the EFA-deficient diet during lactation (group CD) exhibited only marginal delays in reflex acquisition, which was in contrast to the more pronounced effects of maternal pyridoxine deficiency during lactation (18).

A delay in onset of neuromotor skills has been reported in progeny of dams receiving pyridoxine-restricted (9), sodium-restricted [5] or energy-restricted (21) diets during gestation. No such delay was evident in the pups in the present study even if the EFA deficiency was imposed on the dam during both gestation and lactation. Omission of essential fatty acids from the diet of the dam during the final week of pregnancy had no permanent effect on the time the progeny spent standing (cf. group DC, table 2). However, feeding the EFA-deficient diet to the dam during lactation did shorten the time the progeny spent standing (groups CD and DD) during the observation period. A deficiency of pyridoxine in the maternal diet during gestation or lactation also depressed standing activity in the progeny (9, 18). It is interesting to note that lack of linolenic acid in the maternal diet during gestation and lactation decreased the time spent in supported standing of the offspring even though the linoleic acid content of the diet was more than adequate (13). Possibly, lack of linolenic rather than linoleic acid is responsible for the shorter periods spent in standing by pups suckling dams fed the EFA-deficient diet during lactation in the present study.

Spontaneous activity, exemplified by grooming, was adversely affected in progeny of dams restricted in pyridoxine during gestation (9); this was particularly evident at 21 days of age. A similar effect was noted if pyridoxine deficiency were imposed on the dam during lactation (18). Lack of essential fatty acids only during gestation did not adversely affect grooming activity in progeny, whereas deprivation during lactation did curtail this activity.

Lack of protein (17), pyridoxine (9), or sodium [5] in the maternal diet during gestation depressed the total relative activity of the progeny, which may indicate a lower exploratory drive. Maternal energy restriction also impaired exploration in young rats (22). In the present study, pups suckling dams fed the EFA-deficient diet (group DD) exhibited lower total activity than progeny of control dams (group CC) by day 21. In contrast, pups born to dams whose dietary fat was changed at parturition (groups CD and DC) were hyperactive relative to progeny of control dams. This hyperactivity disappeared by day 21 in progeny of dams fed the control diet followed by the EFA-deficient diet (CD) but not in those of dams subjected to the opposite dietary change (group DC). Changing the diet of the dams, rather than feeding the deficient diet per se, may have modified their behavior towards their offspring. Lack of stimulation derived from maternal attention can affect the behavior of the offspring, and rats raised in a stimulus-poor environment are more excitable than those raised in the enriched environment (23).

The developmental and behavioral changes in progeny of rats fed an EFA-deficient diet during pregnancy and lactation were less pronounced than those resulting from deficiencies of a number of other nutrients. Undoubtedly, this was partly due to the limited duration of the prenatal EFA deficiency employed in the current study. However, in instances where a direct comparison of this deficiency with those of other nutrients was possible, e.g. deprivation only during lactation, the lack of essential fatty acids was still less detrimental than a lack of other nutrients. Nevertheless, a brief exposure of the developing fetus to dietary EFA deficiency in the dam did impair development of reflexes, particularly audicular startle, in the newborn. Neuromotor activity was impaired to some extent by maternal EFA deficiency during lactation, but any effects of a dietary deficiency of EFA during gestation appeared to be reversed by the realimentation of the dam during lactation. The results of the current experiment add limited support to the concept that the developing rat brain is affected by deficiencies in the diet of the dam during pregnancy and lactation. On the basis of the parameters investigated, the vulnerability of the brain to a deficiency of essential fatty acids during this developmental period is not as great as its vulnerability to lack of a variety of other nutrients.

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