Influence of Maternal Dietary Fat upon Rat Pups

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SUMMARY

Mother rats were fed purified rations containing different fats during gestation and lactation and at 1 day after parturition. Litter sizes were reduced to 2 male and 2 female pups. The behavior and the brain chemical composition of these selected pups were compared with similarly selected pups from dams fed a commercial ration. All offspring were fed a commercial ration after weaning.

Pups from dams fed 20% safflower oil were similar to controls. Feeding 20% cocoa butter to dams resulted in pups with reduced exploratory activity and with a rapid learning performance in a T-maze, employing the aversive stimulation of an electrical shock. Feeding dams a fat-free ration produced pups which had reduced rates of growth, small brains at 2 months of age, and low brain concentrations of cholesterol, DNA, and RNA.

The stage of development of a rat brain at weaning may be compared to that of a human infant at 20 months of age. The rate of brain development in a rat reaches its peak about 10 days of age, whereas the human brain development has its most rapid growth before birth, with the rate leveling off at about 20 months of age.[9] Since the rat can be easily reared under laboratory conditions and is adaptable to behavioral testing, information derived from rat studies may have value in studies of human beings.

Mammalian brains contain a considerable amount of lipid which is of known importance in brain function. Thus, any dietary manipulation which alters brain lipid might be expected to alter brain function. Protein or protein-calorie deprivation during the first 3 weeks of life in rats resulted in reduced synthesis of brain lipids and reduced brain cholesterol concentrations.[25] Undernourishment of infant rats and swine slowed the myelination process and reduced brain concentrations of lipid and DNA.[8]

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The maternal dietary fat influences the fetal lipids. In guinea pigs, maternal dietary fats influenced maternal blood fatty acids, and fetal blood fatty acids were similar to those in maternal blood.[13] The fetus is dependent upon the maternal diet for essential fatty acids, and linoleic acid has been found to pass the placental barrier in rats[15,21] and in monkeys.[13] When linoleic acid labeled with carbon-14 was injected into gestating rats, the labeled acid was found in the fetal brain phospholipids, indicating that this acid was able to pass the placental barrier and the blood-brain barrier.[7]

A maternal essential fatty acid deficiency may have a lasting influence upon the development of the offspring. Chicks hatched from hens with an essential fatty acid deficiency had retarded growth and difficulties in locomotion and in maintaining equilibrium.[18]

Varying the dietary levels of linoleic and linolenic acids in mother rats influenced the fatty acid composition of the liver, muscle, and serum of the pups at 21 days of age, but the brain fatty acid contents were relatively unaffected.[1] Although maternal dietary fatty acids may enter the brain, the ratio of fatty acids within the brain is apparently closely controlled.

The purpose in the present study was to determine if maternal dietary fats with widely different fatty acid contents or if fat-free rations during gestation and lactation would influence the development and behavior of rat offspring.

Materials and Methods

General Methods—Female Charles River rats were mated with males of the same strain and fed rations by treatments as follows during gestation and lactation: group I—a commercial ration;[a] group II—a purified fat-free ration; group III—a purified fat-free ration containing 20% cocoa butter; and group IV—a purified ration containing 20% safflower oil. The purified ration contained 20% protein and the essential vitamins and minerals, and in group II, corn starch was used in place of fat. At 1 day after delivery, the litter size was adjusted to 2 males and 2 females by random selection. The selected pups were weaned at 3 weeks of age and fed a commercial ration during the remainder of the experimental period. Details on the purified rations, environmental conditions, gestational and lactational observations, and brain chemical composition of the dams and discarded neonates were reported previously.[4]

The selected pups were housed 2 littermates of the same sex in a cage; feed and water were available ad libitum. Environmental conditions were controlled to room temperature (24 to 26 C) and a moderate humidity (45 to 55%). The animal rooms were lighted from 7:00 AM through 7:00 PM.

[a] Purina Laboratory Chow, St Louis, MO.

The data were analyzed statistically,[a] employing the analysis of variance technique. In addition, the T-maze responses were fitted to prediction equations (linear, quadratic, or cubic regression equations), and the equations were used to generate smooth curves for Figures 1 through 6.

Developmental Observations—Litters were observed each day during lactation for the day on which a phenomenon appeared for the first time. Usually, an entire litter exhibited the attribute on the same day, but in instances where all of the litter did not evidence the attribute simultaneously, the day on which the majority of the litter evidenced the attribute was selected.

Hair growth was identified when the skin appeared white rather than pink. Steady standing was judged when a pup could support itself without assistance and without its abdomen touching the cage floor for 30 seconds or longer; a pup was given a single gentle nudge each day until steady standing occurred. Walking was recorded when a pup proceeded unassisted in a relatively straight line of at least 15 cm without the abdomen dragging on the cage floor after a single gentle nudge; a nudge was administered each day until the phenomenon was noted; and locomotion before walking was in a circular path transcribed by the uncoordinated movement of the appendages with the abdomen dragging. Self grooming was recorded when the pups made the first consistent efforts to clean themselves. Body weights were determined once a week, starting at 1 day of age.

Behavioral Observations—Behavioral testing of the pups was initiated on the nearest Monday to 4 weeks of age, and was conducted each weekday for 4 weeks for a total of 20 days of testing.

Exploratory activity was assessed for each pup on each day of testing, using an activity meter[b] set at full sensitivity to record both horizontal and vertical movements. The pup was placed in an open-top plastic box (18 by 25 by 15 cm) which was on the surface of the activity meter. One minute was allowed for the influence of the operator's hand to dissipate and for the rat to acclimate to the box. Thereafter, activity was recorded for 5 minutes.

Learning performance was evaluated in a T-maze, with the negative stimulation of an electrical shock for each pup on each day of testing for a total of 100 tests/offspring. The T-maze was constructed of clear plastic with rectangular tunnels (8.5 by 12.5 cm) and a floor grid capable of carrying an electrical current to the pups. The body of the T was 60 cm long and the arms totaled 115 cm in length. Boxes were separated from the arms of the T by remotely operated doors, were 30 cm long, and were constructed in a similar manner to the T. The start box was located at the base of the T, the safe box was located at the end of the left arm of the T, and the simulated safe box was located at the end of the right arm of the T. When the floor grid was electrified, in all areas except the safe box, its grid carried approximately 0.3 ma of pulsating current at about 110 v of alternating current.

In T-maze testing, the pup was placed in the start box with the start door closed and all other doors open. The door from the start box to the rest of the maze was then opened, and 5 seconds were allowed for the pup to leave the start box before the electrical current was applied. The current was applied until the pup either reached the safe box or until 30 seconds had elapsed, except as noted in the next paragraph.

An avoidance response was recorded when the pup left the start box before the application of the current and proceeded to the safe box within 40 seconds after leaving the start box. If the pup had not arrived at the safe box within a 10-second period, the current was applied for a maximum of 30 seconds. The time lapse from the door opening to the arrival in the safe box was noted with the avoidance response.

An escape response was recorded when the pup remained in the start box until the current was applied, but then proceeded to the safe box within a 30-second period. The time lapse from the application of the current to the arrival in the safe box was recorded.

A nonescape response was recorded if the pup remained in the start box during the 30-second period, or if the pup left the start box but never proceeded to the safe box. Most pups that did not escape remained in the start box. A few left the start box, but returned to the start box during the application of the current.

Brain Observations—When the pups attained the age of 8 weeks, they were killed by decapitation. The entire brain was removed from the spinal cord at the calamus scriptorius, weighed, and homogenized in distilled water at 0 C, employing a homogenizer with a pestle and a glass tube. The volume of homogenate was adjusted to 25 ml, and aliquots were taken, as described in the next paragraph, with sonification applied before each removal to ensure an equivalent aliquot.

Duplicate 5-ml aliquots were dried to constant weight at 37 C and 30 μm of Hg usually for 48 hours. After weighing, these dried aliquots were extracted 3 times with a chloroform-methanol solution (2:1, v:v) and this latter extract was dried (24 hours at 30 C and 30 μm of Hg) to obtain the weight of the lipid extract. The lipid extracts were dissolved in chloroform and centrifuged to remove debris before being made to a volume of 5 ml for the colorimetric determination of cholesterol by the Liebermann-Burchard reaction.[14]

The DNA and RNA determinations (on duplicate 5-ml aliquots) were done by modifications of the Schmidt and Tannhauser procedure, as previously described.[2,5]

The methods of analyses allowed duplicate determinations of each observation, and these duplicates were averaged.

Results

Developmental Observations—The developmental signs in the selected pups, such as walking and eye opening, were not influenced by the dietary lipid of the dams during gestation and lactation (Table 1).

Although the pups were fed a commercial ration after weaning, the dietary fat of their dams influenced their growth (Table 2). Rats from dams fed the fat-free ration (group II) tended to be smaller at birth and grew more slowly than the others, whereas rats from dams fed the cocoa butter (group III) attained the greatest body weight. Male pups grew more rapidly than females.

Behavioral Observations—The exploratory activity of pups from dams fed cocoa butter (group III) was less than that of other pups (Table 3). Activity was usually less in males than in females.

Maternal dietary fat influenced learning performance of pups, as assessed in the T-maze (Table 4). Pups from dams fed cocoa butter (group III) required fewer trials to avoid 3 consecutive times than the others,

[b] Model S Selective Activity Meter, Columbus Instruments, Columbus, OH.

TABLE 1—Development of Pups from Mothers Fed Different Fats

Group No.	Dietary Fat	No. of litters	Appearance of phenomena on days after birth: Hair growth	Eyes open	Steady standing	Walking	Self grooming
I	Commercial ration	11	4	14	6	8	13
II	Fat-free ration	14	4	14	5	7	12
III	Cocoa butter	12	3	13	4	7	12
IV	Safflower oil	10	4	14	6	9	15

There were no significant differences ($P < 0.05$) between groups.

TABLE 2—Growth of Pups from Dams Fed Different Fats

Group No.	Dietary fat	No. of litters	Sex	Growth (av wt in grams): Body weight on day 1	Weight gain/week, first 3 weeks	Weight gain/week, weeks 3 through 7	Body weight at 7th week
I	Commercial ration	11	Male	7.7	20.3ab	42.7b	239.3b
			Female	7.4	20.9a	29.5d	188.1f
II	Fat-free ration	14	Male	7.3	16.3c	41.0b	220.1d
			Female	6.8	16.0c	29.0d	170.5g
III	Cocoa butter	12	Male	7.7	17.2bc	46.8a	246.6a
			Female	7.1	17.2bc	33.5c	192.7e
IV	Safflower oil	10	Male	7.5	17.8ac	42.0b	229.1c
			Female	7.4	17.4bc	32.0cd	187.7f

Statistically significant differences ($P < 0.05$) were found for weight gains and for weight at 7 weeks of age. Values having the same letter after them were within the same significance level ($P < 0.05$) and were assessed by times; values with different letters were statistically different.

TABLE 3—Exploratory Activity of Pups from Dams Fed Different Fats

Group No.	Dietary fat	No. of litters	Sex	Activity by weeks, av counts daily/5 min: 1	2	3	4	Overall, av counts daily/5 min
I	Commercial ration	11	Male	486b	451b	523bc	458cd	479a
			Female	552a	511ab	525bc	518bc	527a
II	Fat-free ration	14	Male	411c	334cd	346ef	494bc	396b
			Female	480b	546a	557ab	548b	532a
III	Cocoa butter	12	Male	231e	216e	318f	328e	273c
			Female	313d	275de	404de	434d	357b
IV	Safflower oil	10	Male	375cd	293d	468cd	484cd	404b
			Female	401c	368c	609a	627a	504a

Statistically significant differences ($P < 0.05$) were found, and generally the activity increased with time, except in group I. Values having the same letter after them were within the same significance level ($P < 0.05$); values with different letters were statistically different. The overall averages were compared separately, and the weekly averages were compared by weeks.

and several pups from dams fed the commercial ration (group I) or the fat-free ration (group II) never avoided 3 consecutive times during the trials.

Projected avoidance performance was influenced by maternal dietary fat. Male (Fig 1A) and female (Fig 1B) pups from mothers fed cocoa butter (group III) avoided more frequently than others. The time required for avoidance of male pups did not differ much between groups (Fig 2A), but female group III pups generally avoided more rapidly than the others.

The projected escape performance reflected the avoidance performance, since pups not avoiding usually escaped. Feeding cocoa butter to the dams (group III) resulted in pups of both sexes that escaped less than the others because they were avoiding (Fig 3A and B). The time required for group III pups of both sexes to escape was also less (Fig 4A and B).

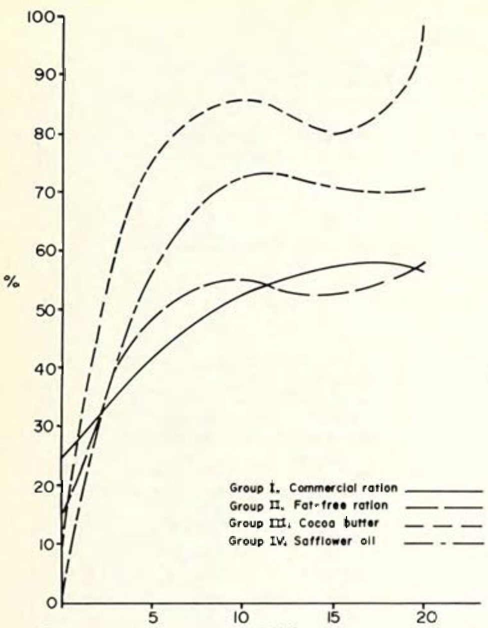
The projected nonescape performance was relatively high in male pups (Fig 5) from dams fed the commercial ration (group I) or from dams fed the fat-free ration (group II). Female pups in group I also had a relatively high incidence of nonescapes (Fig 6).

TABLE 4—T-Maze Avoidance Response of Pups from Dams Fed Different Fats

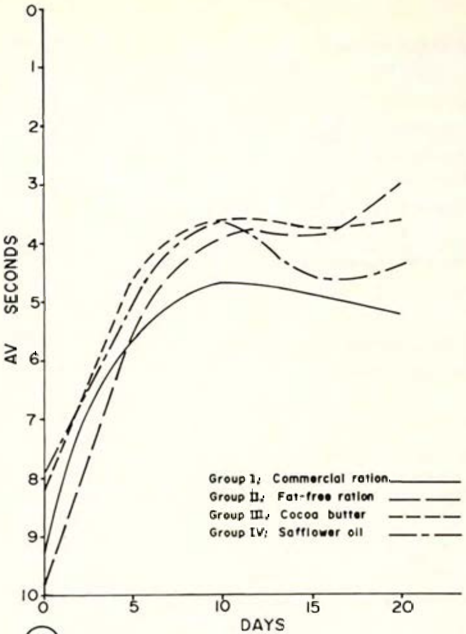
Group No.	Dietary fats	No. in litter	First trial after which offspring avoided 3 consecutive times: Males	Females	No. in group never avoiding 3 consecutive times
I	Commercial ration	11	24abc	18bc	7
II	Fat-free ration	14	15c	27ab	7
III	Cocoa butter	12	14c	14c	0
IV	Safflower oil	10	27ab	30a	0

Statistically significant differences ($P < 0.05$) were found in avoidance. Values were compared both between sexes and between groups. Values with the same letter after them were within the same significance level ($P < 0.05$); values with different letters were statistically different.

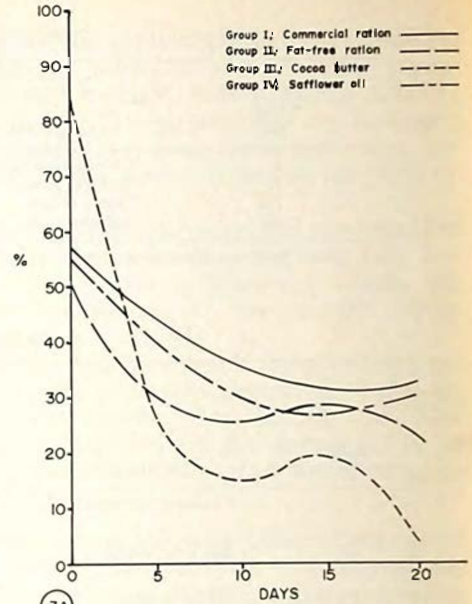
Brain Changes—Maternal dietary fat had a modest influence upon brain composition in pups (Table 5). Feeding dams a fat-free ration (group II) or safflower oil (group IV) resulted in reduced brain weights in pups. However, as brain weight decreased, the ratio of brain weight to body weight increased. Female pups had smaller brains than did males. The dry matter and the lipid concentrations were not materially influenced by the dietary fat of the dam, but the cholesterol con-



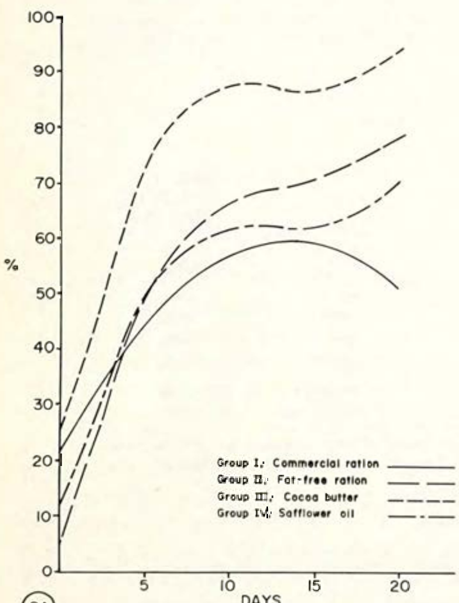
1A



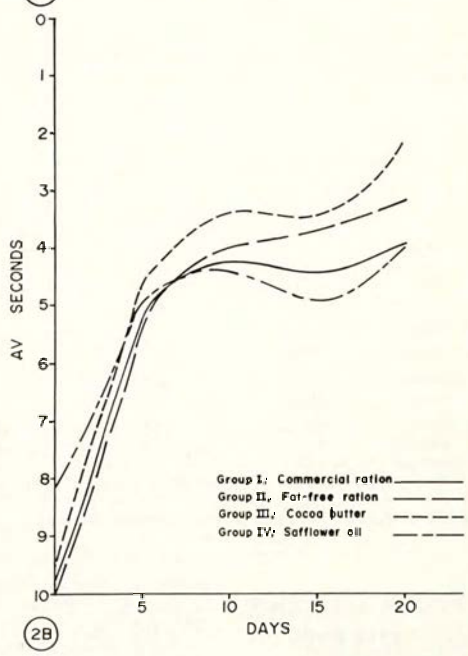
1B



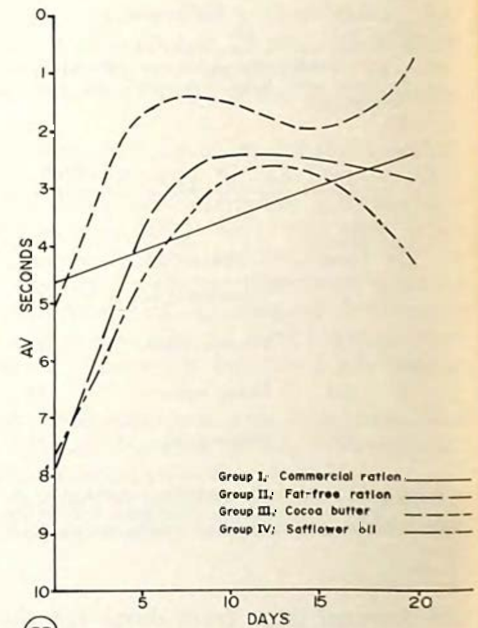
3A



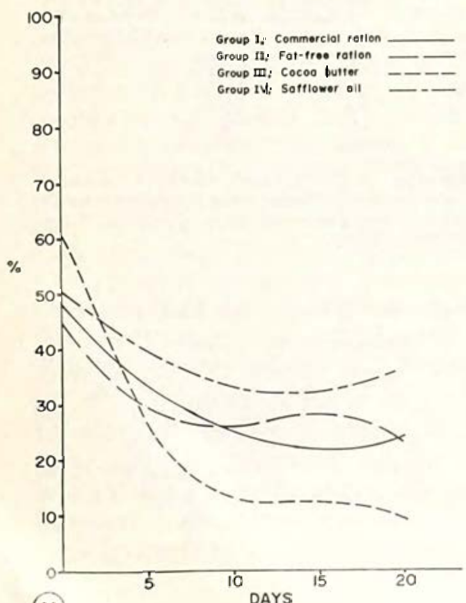
2A



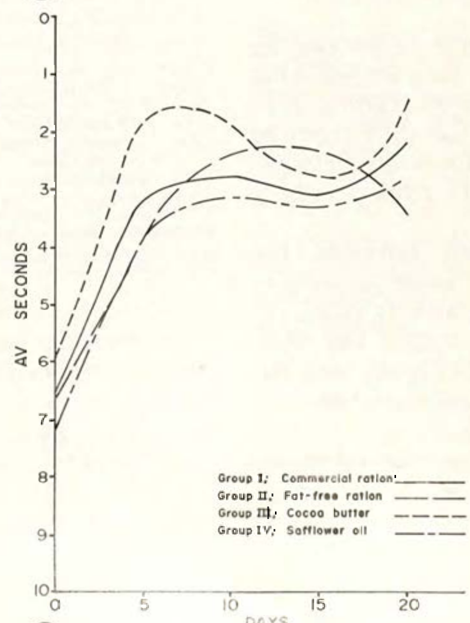
2B



3B



4A



4B

The curves in Figures 1 through 6 were projected from the best fit from the T-maze data. The number of litters is noted in Table 1, and the litter size was adjusted to 2 males and 2 females at 1 day after delivery. Chi-square tests by tests often revealed statistically significant differences ($P < 0.05$) as compared to predicted values as follows: Figures 1A and 2A, the avoidance response was greater for group III males and females; Figures 2A and B, the avoidance time was less for group III males and females; Figures 3B and 4B, the escape time was less for group III males and females; Fig 5, the nonescape response was less for males in groups III and IV; and Fig 5, the nonescape response was greater for females in group I.

Fig 1A—Avoidance response by male pups.
B—Avoidance time by male pups.
Fig 2A—Avoidance response by female pups.
B—Avoidance time by female pups.
Fig 3A—Escape response by male pups.
B—Escape time by male pups.
Fig 4A—Escape response by female pups.
B—Escape time by female pups.

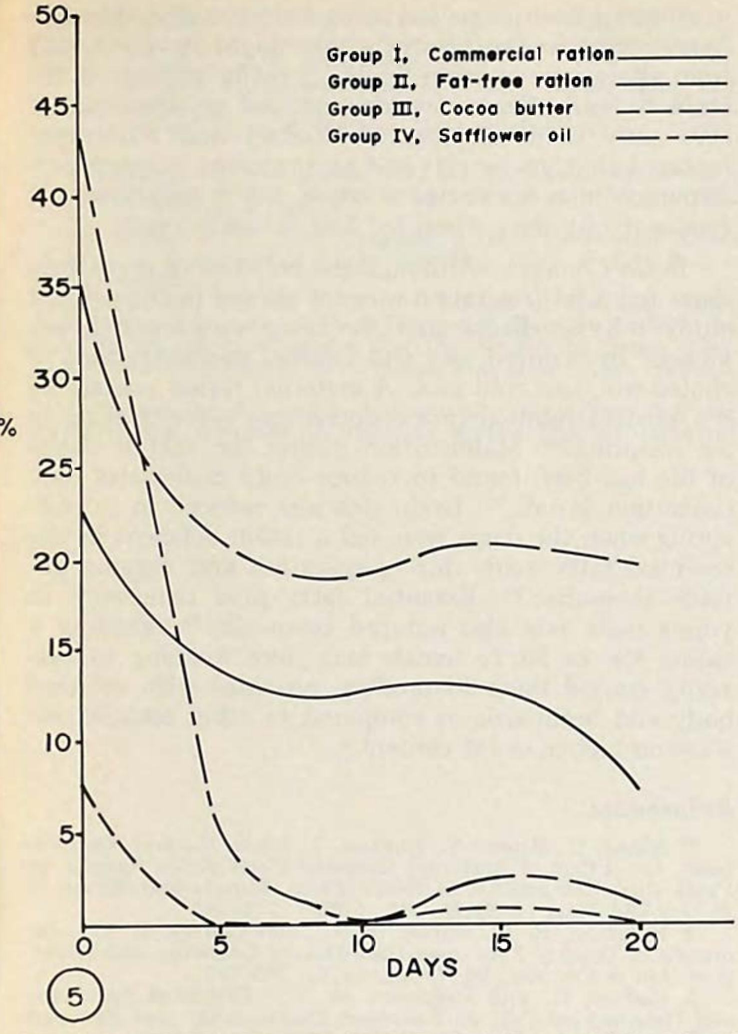


Fig 5—Nonescape response by male pups.

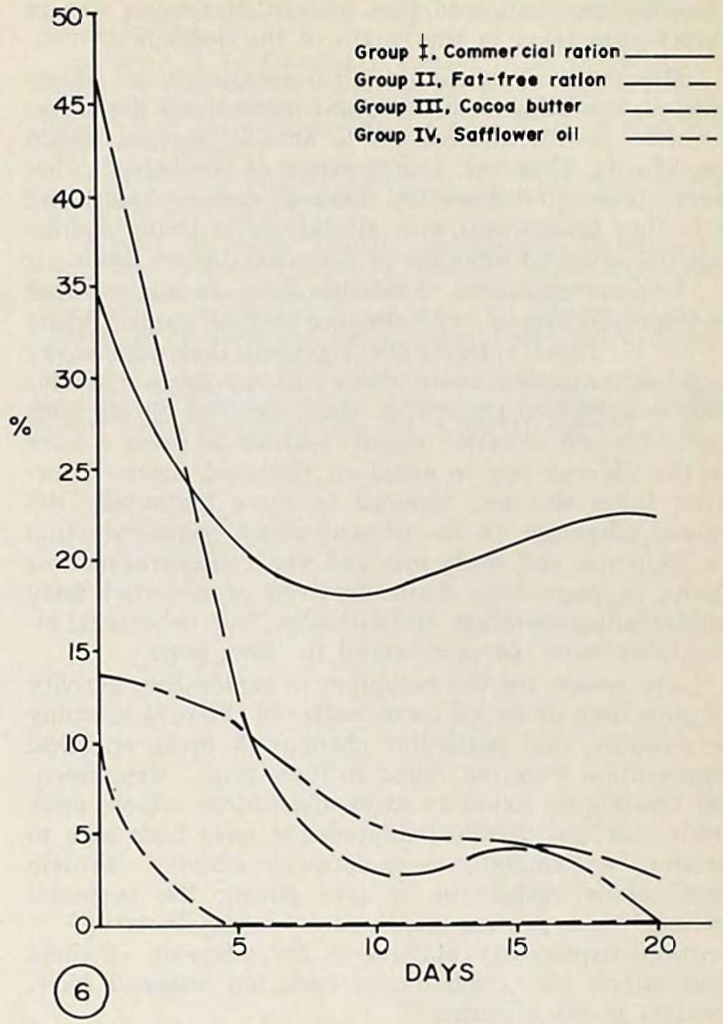


Fig 6—Nonescape response by female pups.

centration tended to be lower in group II pups. The lowest DNA and RNA concentrations were found in pups in group II, but the RNA to DNA ratios were similar in all groups and sexes.

Discussion

Developmental Observations—A ration deficient in essential fatty acids was fed during both growth and lactation in the present study, and both birth weights and growth of pups were depressed. Apparently, the period during which the ration is deficient is of importance in determining the duration of the growth-depressing effect. When dams were fed fat-deficient rations during gestation but not during lactation, pups had a low birth weight, but their weights at weaning and thereafter were similar to those of sufficient controls.[4] A permanent reduction in growth of offspring resulted when dams were fed a deficient ration 1 day before parturition and during lactation, and when the rat offspring were fed a deficient ration for varying periods thereafter.[12] A similar study, where the deficient ration was fed to dams during the last 5 days of gestation, during lactation, and for varying periods to

TABLE 5—Brain Analyses of Pups of Dams Fed Different Fats

Group No.	Dietary fat	No. of litters	Sex	Wet weight (av g)	Wet weight/ body weight (av %)	Dry weight/ wet weight (av %)	Lipid weight/ wet weight (av %)	Choles-terol, av mg/g wet weight	DNA, av mg/g wet weight	RNA, av mg/g wet weight	RNA/ DNA
I	Commercial ration	11	Male	2.060a	0.85c	20.8	8.8	16.9ab	1.27bc	3.28b	2.65
			Female	1.954b	0.98b	21.2	9.7	16.9ab	1.29bc	3.33b	2.62
II	Fat-free ration	14	Male	1.937b	0.87c	21.2	9.3	16.5bc	1.09d	3.17c	2.97
			Female	1.875c	1.09c	22.1	9.3	16.0c	1.10d	3.18c	2.95
III	Cocoa butter	12	Male	2.042a	0.84c	21.3	9.6	17.7a	1.24c	3.35b	2.82
			Female	1.902bc	1.02ab	21.1	9.5	16.9ab	1.37ab	3.47a	2.64
IV	Safflower oil	10	Male	1.942b	0.86c	21.6	9.4	17.2ab	1.40a	3.49a	2.58
			Female	1.898bc	1.03ab	21.8	9.7	16.8bc	1.41a	3.49a	2.59

Statistically significant differences ($P < 0.05$) were found in brain weight, brain weight as related to body weight, cholesterol concentration, DNA concentration, and RNA concentration. Values with the same letter after them were within the same significance level ($P < 0.05$); values with different letters were significantly different.

the offspring, indicated that growth depression was in direct proportion to the length of the deficiency.[24]

Behavioral Observations—Interpretation of alterations in the behavior of rats, and particularly the extrapolation of this information to another species, would be difficult. However, a comparison of the behavior between pups from dams fed different dietary lipids, and a further comparison with alterations in brain chemistry, indicated an influence of maternal dietary lipids.

Undernourishment of rats during gestation, resulted in improved learning performance in their pups (Smart et al[22]). These authors also reported that postnatally deprived pups were more efficient in running a maze for food reward, and pups from dams deprived during both gestation and lactation rapidly learned to press a lever in the Skinner box to avoid an electrical shock. However, brain size was reduced in these perinatally deprived offspring. In the present study, some reduction in brain size and brain DNA and RNA concentration was found in pups from dams deprived of essential fatty acids during gestation and lactation, but behavioral alterations were not pronounced in these pups.

The reason for the reduction in exploratory activity of pups from dams fed cocoa butter in the present study is puzzling, and particular changes in brain chemical composition were not found in these pups. Experimental treatments found to cause deleterious effects upon brain size and chemical composition have been seen to produce a reduction in exploratory activity. Protein and calorie restriction in rats during the perinatal period[11] and protein deprivation during lactation[16,17] reduced exploratory activity in rat offspring. Protein and calorie restriction during lactation lessened social contact in rat offspring.[10]

Although exploratory activity was apparently unaffected in pups from dams fed a fat-free ration in the present study, Musty and Chipault[19] found a modest increase in this activity when young rats were fed a ration containing no fat except for a small amount of linoleic acid. The difference in findings might be explained by the different stages of development when the experimental rations were fed, and the latter study was not concerned with the essential fatty acid deficiency produced by the fat-free ration in the present study.

Learning performance in the T-maze was not appreciably influenced in offspring from dams fed a fat-free ration in the present study, in contrast to the findings of others. Caldwell and Churchill[4] fed rats a fat-free ration during gestation, and noted reduced learning performance of the offspring in a T-maze when employing a food reward. Paoletti and Galli[20] fed young rats a ration deficient in the essential fatty acids for 90 days and found fewer conditioned avoidance responses in a shuttle box. Rats weaned prematurely had a reduced learning performance at 1 year of age; apparently, this change was caused by a reduction in fat intake in these rats during lactation, since the feeding of a high-fat ration from 18 through 30 days of age prevented the reduction in learning performance in early-weaned rats.[13]

The alterations in learning performance observed in offspring from dams fed cocoa butter during the perinatal period in the present study might have resulted from alterations in brain lipids. A major portion of the brain is lipid. Brain development and myelination occurs early in development.[25] Caffrey and Patterson[3] reported that young rats had an improved learning performance in a water maze when fed a saturated fat (coconut oil) than when fed less saturated fats.

Brain Changes—Although the behavior of pups from dams fed a fat-free ration was not altered in the present study, adverse effects upon the brain were found as evidenced by reduced size and lowered concentrations of cholesterol, DNA, and RNA. A maternal ration containing 8% protein resulted in a reduced DNA concentration in rat offspring.[26] Malnutrition during the first 3 weeks of life has been found to reduce brain cholesterol concentration in rats.[25] Brain size was reduced in rat offspring when the dams were fed a ration deficient in the essential fatty acids during gestation and varying periods thereafter.[24] Essential fatty acid deficiency in young male rats also reduced brain size.[12] Feeding a ration low in fat to female rats from weaning to maturity caused them to produce neonates with reduced body and brain size, as compared to other females fed a ration higher in fat content.[6]

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