JOURNAL OF THE AMERICAN GERIATRICS SOCIETY
Vol. XXIV, No. 7

Printed in U.S.A.

Free Radical Theory of Aging: Effect of Dietary Fat on Central Nervous System Function*

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ABSTRACT: Free radical reactions have been implicated in aging. A rise in the level of random free radical reactions in a biologic system might have a greater effect on the central nervous system (CNS) than elsewhere, partly because of the presence of glial cells and the unique connections between neurons. To evaluate this possibility, some animal experiments were conducted. The initial experiment involved old male Sprague-Dawley rats fed (since shortly after weaning) with semisynthetic diets characterized by fat differing in amount or degree of unsaturation. The number of errors made in a Hebb-Williams maze was determined and found to be higher as the amount or degree of unsaturation of the fat was increased. Likewise rats aged 6 and 9 months, fed semisynthetic diets containing 20 percent by weight of lard, oleinate, or safflower oil+α-tocopherol performed significantly better in a discrimination learning situation (Skinner box) than did rats fed a diet containing 20 percent by weight of safflower oil. The diets employed in these studies did not have a significant effect on the mortality rates. These results are compatible with the possibility that enhancing the level of lipid peroxidation has an adverse effect on the CNS, out of proportion to the effect on the body as a whole, as measured by the mortality rate.

Chronic organic brain syndrome (COBS) is a major health problem (1, 2). In this disease the central nervous system (CNS), at least that part involved in higher functions, can be regarded as aging faster than the body as a whole, becoming "old" to the point where the individual is deprived of intellectual and emotional life for a significant period before death. The incidence of COBS rises rapidly with advancing age, beginning at about age 70 (1). Since the median age of the 65+ group is increasing (3), marked increases can be expected in the number of persons with COBS.

COBS patients can be divided into three large groups on the basis of the major brain lesions: a) senile plaques 47 percent; b) vascular disease 30 percent; and c) mixed "a" and "b" 23 percent (4). Recent work indicates that degenerative changes in aging dendrites (5) may also be involved in COBS.

The etiology of COBS is unknown. Free radical reactions may be involved in the pathogenesis. This class of chemical reaction (6, 7), ubiquitous in biologic systems (8, 9), has been implicated in the degradation of such systems. On this basis, one possible means of decreasing the rate of degradation in the central nervous system and elsewhere, would be to decrease the ingestion of dietary components that might reasonably be expected to participate significantly in more-or-less random free radical reactions in vivo. Fat is such a dietary factor (10), and it is a major component of most human diets. The fatty-acid moieties present in dietary fat differ markedly

* This investigation was supported in part by grants from the NIH (HE-06979) and the U.S. Department of Agriculture.

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in the ease with which they are peroxidized (11), i.e., react with molecular oxygen; peroxidation of a polyunsaturated fatty acid such as linolenic acid proceeds much more rapidly than that of stearic acid, a saturated fatty acid. Thus, decreasing the amount or degree of unsaturation of dietary fat might be expected to result in a decreased rate of biologic degradation.

In general, dietary fat might be expected to have an effect on the CNS similar to that on the body as a whole. However, the CNS response may be greater than elsewhere in the organism because the neurons are fixed postmitotic cells with unique connections with other neurons (12), while exchange with the capillaries, in contrast with parenchymal cells elsewhere, is modified by the presence of glial cells. The foregoing characteristics could conceivably make the CNS more susceptible than the rest of the body to accumulative deleterious changes which in turn could be reflected in CNS degradation becoming clinically evident for varying periods before death, the duration of disability being different for each individual.

To evaluate this possibility—that increases in the level of more-or-less random free radical reactions secondary to increased lipid peroxidation might have a selective adverse effect on the CNS—two experiments were conducted in which the relative efficiency in a learning situation was used as a measure of CNS deterioration. In the first study the effect of the amount or degree of unsaturation of dietary fat on the mortality rate for rats was determined, as well as the maze-learning abilities of the animals when old. The second experiment was designed to assess the influence of dietary fats and of one antioxidant, vitamin E, on the learning behavior of relatively young rats, aged 3, 6 and 9 months; operative behavior was evaluated using a discrimination learning paradigm.

EXPERIMENT 1

Method

Four hundred Sprague-Dawley male rats were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts shortly after weaning and caged, 4 per cage (stainless steel, $11\frac{1}{2} \times 18\frac{1}{4} \times 6\frac{1}{2}$ inches). The rats were maintained in an air-conditioned room at 76–78°F at a humidity of 50–60 percent. Cages were cleaned 2–3 times per week. The bedding consisted of sterilized shredded corn cobs (San-i-Cel, Paxton Processing Company, Paxton, Illinois). At age 2 months the rats were divided at random into groups and given semisynthetic diets (13) containing 5, 10 or 20%w (percent by weight) of lard, olive oil, corn oil, safflower oil or distilled triglycerides of menhaden oil (Technological Laboratory, Bureau of Commercial Fisheries, Fish and Wildlife Service, U. S. Department of the Interior, Seattle, Washington). There were 28 rats in each of the 5 percent and 10 percent groups and 24 rats in each of the 20 percent groups. The lard (antioxidant-free), olive oil, corn oil and safflower oil were all edible grade, marketed for human consumption.

The composition of the 5%w lipid diet has been published (14); 10 and 20%w lipid diets were prepared at the expense of glucose monohydrate. These diets meet the nutritional requirements of rats (15). Although the amount and type of dietary fat are the only significant variables in these diets, they are not the only variables. Thus, the thiamine requirement of a diet increases with the percentage of carbohydrate. α-Tocopherol acetate was added in the proportion of 20 mg per 100 gm of finished diet, to obviate the possibility of vitamin-E deficiency.

The 5%w, 10%w and 20%w fat diets contained 408, 433 and 483 calories, respectively, per 100 gm. The diets were fed isocalorically; for each gram of diet fed the 20 percent groups, the 10 percent groups received 1.12 gm, and the 5 percent group 1.19 gm. Food consumption was determined once a month for a period of one week; the average amount of food eaten per rat per day in the dietary groups eating the least during the week period was fed to all the groups during the ensuing month. Despite the foregoing, the body weights tended to increase with the level of dietary fat. Judging from the animals' appetites, all the diets were about equally palatable.

The diets were made up once a week and stored in glass jars prior to use; the safflower and menhaden oil diets were kept in a deep-freeze, while the other diets were stored in a cold-room at refrigerator temperature. Samples of the diets were analyzed several times for peroxides by the iodiometric method (AOCS Official Method Cd 8–53), but no peroxides were found.

The rats, ear-coded, were weighed and counted each month. No adjustments were made when deaths decreased the number (originally 4) in a cage.

Learning behavior was assessed at age 32–35 months, with use of an adaptation (16) of the Hebbs-Williams closed field maze (17). In this

maze the positions of the start and goal boxes remain constant while the pathway to the goal box is determined by the positions of adjustable barriers. The field is divided into 36 alternate black and white 5-inch squares. Entrances to any squares which were not in the direct path between the start and goal boxes were counted as errors. The rats were tested under 23-hour food deprivation; reinforcement was accomplished with 45-mg Noyes pellets (P. J. Noyes Co., Lancaster, New Hampshire). The maze floor was covered by a 5/8-inch layer of water which served as an additional motivating factor. This supplementary aversive stimulus was found to be necessary for the elicitation of maze-running behavior in these old rats. The animals were given 8 trials in each specific maze configuration. Six practice mazes were run by each rat, to adapt him to the apparatus and handling and to establish the habit of eating in the goal box. The rats were habituated to the test situation between 29 and 31 months of age. Testing in the maze occurred between 32 and 35 months of age.

Results

The percentage surviving, and average weight for each of the 15 dietary fat groups are shown in Table 1 as a function of age in months. The amount or degree of unsaturation did not have a significant effect on the mortality rate (13). However, the mortality rates for the 20 percent groups were somewhat higher than those for the corresponding 5 percent and 10 percent groups. The mortality rates for the 20 percent groups tended to increase, except for the menhaden oil rats, with increasing unsaturation of the dietary fat. The mortality rates for the menhaden oil groups were lower than had been expected from the degree of unsaturation of the oil; this same effect was also noted in studies at the same time with C3H and Swiss mice (13). The unexpectedly relatively low mortality rates for the menhaden oil groups possibly may have been a result of the less ready enzymatic hydrolysis of the 20:5 and 22:6 fatty acids present in menhaden oil (18) (so that the lipid actually absorbed from the intestinal tract may have been more saturated than that ingested), and of a preferential utilization of highly unsaturated fatty acids for energy production (19).

The maze data are presented in Table 2. The maze study was conducted at a time when the mortality rates for the 15 dietary groups were high, so many rats did not live to complete all the mazes. For this reason the data are divided into three parts; the top third of the table show data for rats that completed mazes 1-4, the

TABLE 1
Effect of Dietary Fat on Mortality Rate (Sprague-Dawley Male Rats)

Fat in Diet, % by Weight	Age of Rats (mos.)	Lard		Olive Oil		Corn Oil		Safflower Oil		Menhaden Oil	
		W[1]	S[2]	W	S	W	S	W	S	W	S
		N = 28[3]		N = 28		N = 28		N = 28		N = 28	
5% w groups	28	625	71.4	633	64.3	613	64.3	633	67.9	644	57.4
	30	566	50.0	600	50.0	556	60.7	569	46.4	664	39.3
	32	512	42.9	557	35.7	535	53.6	535	42.9	550	28.6
	34	508	21.4	508	25.0	510	35.7	479	39.3	509	21.4
	36	515	14.3	502	14.3	497	21.4	467	21.4	522	10.7
	38	650	3.6	–	0.0	590	7.2	518	14.3	373	3.6
		N = 28		N = 29		N = 28		N = 28		N = 28	
10% w groups	28	610	53.6	581	72.4	634	60.7	612	64.3	664	67.9
	30	602	32.1	606	55.2	636	53.5	612	50.0	614	57.1
	32	541	28.6	545	44.8	607	39.3	551	39.3	582	50.0
	34	543	17.9	549	31.0	550	32.1	614	39.3	561	39.3
	36	556	3.6	544	13.8	475	17.9	581	17.9	574	10.7
	38	–	0.0	554	3.5	599	3.6	540	7.2	–	0.0
		N = 24		N = 24		N = 23		N = 24		N = 24	
20% w groups	28	662	70.8	704	58.3	762	47.8	678	29.2	679	62.5
	30	626	45.8	659	37.5	689	13.0	731	12.5	718	54.2
	32	612	33.7	575	25.0	646	10.5	668	12.5	638	29.2
	34	582	25.0	546	12.5	574	10.5	561	12.5	673	20.8
	36	504	12.5	–	0.0	715	5.3	662	4.2	735	12.5
	38	532	4.2	–	0.0	–	0.0	–	0.0	540	4.2

[1] = Weight in grams.
[2] = Percentage of original group still alive.
[3] = Initial number of rats.

TABLE 2
Effect of Dietary Fat on Average Number of Errors in a Modified Version of the Hebb-Williams Closed-Field Intelligence Test (Sprague-Dawley Male Rats)

% Fat (by Weight) in Diet	Dietary Fat				
	Lard	Olive	Corn	Safflower	Menhaden
			Mazes 1-4: (Age 32-33 months)		
5%w	17.8±5.2[1] [12]	20.1±5.6 [10]	23.2±8.4 [13]	21.8±6.4 [11]	25.5±9.8 [7]
10%w	19.5±7.2 [6]	20.6±6.0 [11]	22.6±6.3 [10]	22.9±6.4 [9]	23.1±7.9 [12]
20%w	21.3±6.6 [8]	21.3±6.5 [6]	17.5±7.0 [2]	22.3±7.2 [3]	24.2±5.7 [5]
			Mazes 1-8: (Age 32-34 months)		
5%w	17.0±6.6 [7]	19.5±4.6 [5]	21.8±7.1 [10]	20.0±7.2 [9]	24.8±10.3 [5]
10%w	17.9±7.3 [6]	21.0±6.0 [7]	21.1±7.6 [8]	22.1±7.7 [6]	24.3±7.5 [9]
20%w	21.3±6.5 [7]	19.5±7.2 [3]	19.4±7.0 [2]	23.7±8.0 [2]	23.6±5.3 [3]
			Mazes 1-12:(Age 32-35 months)		
5%w	18.6±7.1 [5]	21.8±6.0 [4]	22.9±7.0 [7]	19.1±7.3 [6]	26.7±10.3 [4]
10%w	20.5±9.8 [4]	24.9±8.7 [6]	24.1±8.8 [6]	25.0±7.4 [4]	25.8±7.4 [8]
20%w	22.0±7.8 [5]	21.7±6.9 [2]	24.8±8.2 [1]	28.4±10.4 [2]	27.0±7.5 [3]

[1] = Standard deviation.
[2] = Number of rats.

middle third for those finishing mazes 1-8, and the bottom third for those completing all 12 of the standardized mazes. Each figure in the table is the average number of errors per maze ± the standard deviation made by each rat completing the maze series; thus, each of the 12 rats in the 5%w lard group that completed mazes 1-4 made an average of 17.8 ± 5.2 errors (i.e. the total number of errors made in running a given maze 8 times) for each of the four mazes.

In general the number of maze errors increased both with an increase in the amount of dietary fat and an increase in unsaturation of the dietary fat. Statistical analysis (variance) of the data for the animals that completed all 12 mazes—combining the data of the 10 percent and 20 percent groups because of the small number of rats in the latter—showed that this conclusion was valid at $P < 0.05$.

EXPERIMENT 2

Six-Month-Old Rats

Method

Male and female Sprague-Dawley rats were obtained at weaning and caged and maintained as in the initial study. At the age of 1 month the females were given semisynthetic diets (13) containing either 5 or 20%w (percent by weight) of lard, oleinate, safflower oil, or safflower oil plus 20 mg of α-tocopherol acetate per 100 gm of finished diet; the lipids were of edible grade. The fatty acid composition of the three dietary lipids is shown in Table 3. When the rats were 3 months old, one male of the same age as the females and fed prior to mating on a commercial pelleted diet (Rockland, Teklad, Inc., Monmouth, Ill.), was placed in a cage with 4 females for a period of 7 days and then removed. The offspring at 23 days of age, were weaned, sexed, caged 4 per cage, and maintained on the same diet as their mothers.

TABLE 3
Dietary Lipids: Fatty Acid Analysis

Carbon Number	Safflower (%)	Lard[d] (%)	Safflower-Olive (%)
10			0.1[a]±0.1[c]
12			0.2±0.1
14	0.5[a]±0.3[c]	1.7[b]±0.1[c]	0.4±0.3
16	6.7±0.6	26.5±0.2	5.8±0.6
16:1ω7		2.1±0.4	0.1±0.05
18	1.6±0.3	12.1±0.7	1.2±0.1
18:1ω9	11.1±0.05	49.8±1.7	75.5±0
18:2ω6	79.7±0.1	10.3±0.2	15.8±0.9
20 or 22 acids	0.1±0.05	2.4±1.4	

[a] The average of two samples taken at a 6-month interval from the dietary oil.
[b] The average of three samples taken at intervals within a year.
[c] Standard error of the mean.
[d] The lard contained a trace amount of 22:6ω3 as the free acid.

At 6 months of age, 4 male rats from each of the groups—20%w lard, 5%w lard, 20%w safflower oil + vitamin E, and 20%w safflower oil—were drawn randomly for behavior testing. The operator did not know the composition of the diets; the dietary groups were simply labeled A, B, C, and D: A represented lard 20%w; B, lard 5%w; C, safflower oil 20% + vitamin E; and D, safflower oil 20%w.

The rats were maintained at approximately 80 percent of their free-feeding body weights and tested under conditions of 48-hour food deprivation. Testing was conducted in a Skinner box

(Lehigh Valley operant chamber; Lehigh Valley Electronics, Lehigh Valley, Pa.). This device was provided with two manipulanda (bars) which operated a food delivery magazine placed midway between the bars. Noyes reinforcement pellets (40 mg) were delivered when appropriate responses were emitted. Contingencies between responses (bar depression), cue lights (on or off) and the delivery of reinforcements were automatically controlled. A cue light was available over each bar. Animals were first placed in the testing chamber for 30 minutes with both bars set to deliver a food pellet after one press. This procedure was continued for 5 sessions conducted every other day. Animals which had not by that time begun to bar-press spontaneously were shaped by hand. After that time the reinforcement contingency was switched to FR-4 (the rat received a reinforcement consequent to emitting a total of 4 bar presses on one or both of the bars). These tests lasted 15 minutes and were conducted every other day. Three such test sessions were conducted for each animal. There were no differences between the groups under FR-4 testing.

After FR-4 training, a discrimination procedure was introduced in which only responses on the bar over which a cue light was lighted would result in reinforcement. After every correct response there was a 50:50 probability that the light would switch to the other bar. The tests were also of 15 minutes' duration and conducted every other day. Three such tests were conducted.

Results

The average number of correct responses ± standard deviation per rat for the 3 tests conducted under FR-4 discrimination for the 4 groups are tabulated in Table 4. Group D (safflower 20%w) exhibited significantly fewer correct responses ($P < 0.01$, Mann-Whitney U test). There were no significant differences among the other groups. Group D (safflower oil) exhibited a significantly lower percentage of correct responses ($P < 0.05$) on tests 2 and 3 but not on test 1 (Table 4); there were no significant differences among the other groups.

To confirm these data, two additional groups of 4 rats each, labelled A and B, were given to the operator for evaluation. These rats were females, aged 9 months, and they had been given the semisynthetic diets since age 2 months. As in the case of the 6-month-old rats, there were no differences among groups under FR-4 testing. The number and the percentages of correct responses under FR-4 discrimination are shown in Table 5. Group B (20%w safflower) exhibited significantly fewer correct responses than Group A (20%w lard) ($P < 0.01$). The percentage of correct responses by Group B was significantly lower on the first test ($P < 0.01$) but the differences were not significant for tests 2 and 3.

Three-Month-Old Rats

Method

This study employed 3-month-old male rats. The rats, born of mothers from the groups re-

TABLE 4
Effect of Dietary Fat on Discrimination Learning at Age 6 Months (Sprague-Dawley Male Rats)*

Number of Correct Responses

Diet†	No. of Correct Responses ± S.D. Test 1	Test 2	Test 3
A. Lard, 20%w	359±283	463±152	350±350
B. Lard, 5%w	291±171	409±103	398±102
C. Saff., 20%w + 20 mg vit.E	314±179	519±177	705±292
D. Saff., 20%w	90±49‡	118±80‡	104±76‡

Percent Correct Responses

	% Correct Responses ± S.D. Test 1	Test 2	Test 3
A. Lard, 20%w	56±24	53±5	65±16
B. Lard, 5%w	59±12	55±8	60±5
C. Saff., 20%w + 20 mg vit.E	50±4	53±7	65±6
D. Saff., 20%w	49±7	46±5‡	43±17‡

* Born of mothers receiving the same diets.
† Four rats in each group.
‡ Significantly smaller, $P < 0.01$, than the value for the other three groups.

TABLE 5
Effect of Dietary Fat on Discrimination Learning at Age 9 Months (Sprague-Dawley Female Rats)*

Number of Correct Responses

Diet†	No. of Correct Responses ± S.D. Test 1	Test 2	Test 3
A. Lard, 20%w	166±144	190±88	400±78
B. Safflower, 20%w	16±16‡	145±64‡	162±76‡

Percent Correct Responses

Diet†	% Correct Responses ± S.D. Test 1	Test 2	Test 3
A. Lard, 20%w	59±28	50±20	80±27
B. Safflower, 20%w	26±16‡	61±19	73±25

* Diets started at age 2 months.
† Four rats in each group.
‡ Significantly less, $P < 0.01$, than the value for Group A.

ceiving 20%w lard, 20% safflower oil+vitamin E, or 20%w safflower oil, as well as from mothers fed standard laboratory chow pellets (Rockland), were maintained with the same diet as their mothers. As before, except for the Rockland group, the operator did not know the composition of the diet. The diets were labeled A, B, C, D: Group A, Rockland; Group B, 20%w lard; Group C, 20%w safflower oil+vitamin E; and Group D, 20%w safflower oil.

The animals were tested in the operant chamber in the same manner as in Experiment 2A.

Results

Only in one aspect of the operant behavior testing was there a reliable effect of diet. This influence was on the percentage of rats spontaneously shaping; the values for the Rockland, 20%w lard, 20%w safflower oil+vitamin E, and 20%w safflower oil groups were, 76, 78, 95 and 25 percent, respectively. The 20%w safflower oil group was significantly poorer in this respect than the other three groups ($P < 0.01$). Responding under FR-4, or FR-4−discrimination, did not differentiate among the groups.

The earlier discrimination studies demonstrated that rats aged 6 months and 9 months in the safflower oil group, without the vitamin E supplement, had a severely depressed capacity to deal with the operant situation. With 3-month-old rats this suppression was evident only when the free shaping situation was considered.

DISCUSSION

Dietary fat did not significantly alter the lifespan of rats employed in the initial experiment with the Hebb-Williams maze or of those employed in the second experiment (20) with a Skinner box. However, in both studies, dietary fat modified learning behavior and, by implication, the functioning of the CNS. The results of both experiments are compatible with the possibility that enhancing the level of lipid peroxidation has an adverse effect on the CNS out of proportion to its effect on the body as a whole, as measured by mortality rate. Thus variation in the amount or degree of unsaturation of dietary fat and of factors (e.g., vitamin E) that can modify lipid peroxidation rates, may contribute to the variability in age of onset of evident degradative CNS changes such as senility, above and beyond the variations expected from differences in mortality rates.

The manner in which increases in the amount or degree of unsaturation of dietary fat modifies CNS function is unknown. Several mechanisms may be operating. Neuronal dysfunction may be mediated in part through deleterious effects of dietary fat on the glial cells. The rate of peroxidation of serum and vessel-wall constituents may be increased, leading to a more rapid development of arteriocapillary fibrosis (21). Increased lipid peroxidation in the synaptic areas, areas rich in polyunsaturated fatty acids (22) such as 22:5ω6 and 22:6ω3, could cause damage in a manner similar to that caused by β-hydroxy dopamine (23), or by the anesthetic, halothane (24, 25).

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