Longer Survival Time of Rats Fed Oxidized Vegetable Oils.* (31442)

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In a long term study of the nutritional effects of mildly oidized fats on various parameters, observations of survival rates gave some unexpected results(1). Rats fed fresh olive and cottonseed oils had somewhat shorter survival rates than those given fresh beef fat or chicken fat. Feeding of oxidized olive oil led to a higher survival rate than that of the rats fed the fresh oil. These findings suggested further studies with butter, lard, corn and soybean oils.

Materials and methods. All fats and oils used had been processed for human consumption. The origin of the olive oil, cottonseed oil, beef fat and chicken fat has been reported (1). During the current series, fresh corn oil, soybean oil, lard and butter oil were used. Butter oil, instead of butter, was chosen to avoid admixtures of water and milk products. Part of each fat was aerated at a temperature of 60°C for 40 hours, after which all portions were stored at —20°C. Aeration for 40 hours did not change peroxide values except in one corn oil sample in which it underwent a mild increase to 21.4.† Peroxide numbers of the fats used in the earlier studies have been reported(1). The fats were fed, at a level of 20%, in a diet containing 30% alcohol-washed casein, 44% dextrose, 4% USP XIII #2 salt mixture,‡ 2% cellulose, and supplemented with all known vitamins (1).

Some degree of iodine deficiency was desired because of other aspects of the experimental design. The salt mixture USP XIII contains virtually no iodine. However, the diets as a whole afforded daily iodine intakes of 1.5 to 2.5 μg. In the studies of Levine *et al*(2) this amount suppressed the occurrence of goiters and Nordsiek noted, under similar conditions, hardly any systemic effects in rats(3). In our studies, goiters occurred frequently, as will be reported subsequently, but growth and food intake in the previous studies(1) and again in the current series were what one may expect for normal rats. This suggests that the systemic effects were only mild.

The experiments were carried out on male rats of the Columbia-Sherman strain in the same room and with the same personnel as the earlier study. Pregnant rats, on about the 17th day of gestation, were placed on a diet similar to the experimental diet but with the fat replaced by dextrose plus 2% of linoleic acid.§ Only the male offspring of these rats were saved in order to give them every advantage during suckling. The young were weaned, earmarked, and weighed at 24 days and were reweighed at 27 days, at which time the matching experimental groups were made up. The average weights ± standard errors of these groups were the same as at 24 days and again at 27 days. The rats were housed 2 to a cage on racks holding 4 cages on each of 5 shelves. Each shelf contained one cage each of animals fed fresh and aerated butter and lard. The same arrangements were carried out with the rats fed fresh and aerated corn

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† Peroxide numbers for individual batches of fats before and after aeration respectively were: butter: 7.7 and 7.4, 1.3 and 3.0, 100 and 1.55; lard: 6.0 and 5.7; corn oil: 9.3 and 21.4, 1.7 and 2.6; soybean oil: 1.6 and 1.7.

‡ USP XIII #2 salt mixture has no added zinc, manganese, or copper. However, analysis of the diets for these elements by standard methods together with frequent measurement of food intakes (about 15 g/day) showed that the rats had a daily intake of about 20 μg of manganese, 30 μg of copper, and 200 μg of zinc. In addition, the rats may have consumed unspecified amounts from their drinking water and from the wood shavings used as bedding.

§ Linoleic acid concentrate from Nutritional Biochemicals Inc. which is 75% linoleic acid. Rapid rancidification was prevented by the presence of 20 mg of free d,l alpha tocopherol and 100 mg of d,l alpha tocopherol acetate per kilogram of diet. The diet was prepared once weekly and stored in the refrigerator.

TABLE I. Average Life Spans of Male Rats Fed Various Fresh and Mildly Oxidized Animal Fats and Vegetable Oils.

		No. of rats	Avg life span (days)*
Earlier series			
Chicken fat	Fresh	8	734 ± 39
	Oxidized	11	682 ± 34
Beef fat	Fresh	8	675 ± 67
	Oxidized	10	603 ± 43
Cottonseed oil	Fresh	15	655 ± 33
	Oxidized	18	599 ± 33
Olive oil	Fresh	16	592 ± 37
	Oxidized	16	742 ± 26
Present series			
Butter	Fresh	28	782 ± 22
	Oxidized	28	741 ± 23
Lard	Fresh	30	746 ± 25
	Oxidized	16	714 ± 47
Corn oil	Fresh	14	611 ± 46
	Oxidized	14	708 ± 46
Soybean oil	Fresh	14	673 ± 42
	Oxidized	14	807 ± 32

* ± standard error.

and soybean oils. Thus some of each group were kept at each shelf height in an effort to minimize temperature gradients from higher to lower shelves. The racks were kept in close proximity to each other and rotated periodically in order to minimize variation in light and ventilation. All animals were inspected daily, including week-ends, and autopsies were performed as soon as possible after discovery of a dead animal.

Histological examinations of heart, aorta, lung, liver, adrenals, kidneys, stomach, testes, thyroid, brain, and pituitary, were carried out on sections stained with hematoxylin-eosin after fixation in Bouin's solution.

Results. In Table I are summarized the average life spans of the various groups. For purposes of statistical evaluation, all animals which were still alive at 893 days of age, were given this age. This seemed permissible since it could only minimize differences between groups having few and many survivors.

In both series (Table Ia and b), rats fed fresh vegetable oils did not live as long, on the average, as those fed fresh animal fats. (Fresh chicken fat *vs* fresh olive oil, P less than .05; fresh butter and fresh lard *vs* fresh corn oil and fresh soybean oil, P less than .01.) Oxidation of 3 of the vegetable oils increased the survival rates approximately to those of the rats fed fresh animal fats. (Oxidized olive oil *vs* fresh olive oil, P less than .01; oxidized soybean oil *vs* fresh soybean oil, P less than .02.) Oxidation of the animal fats led to shorter life spans. Lea and Parr reported a similar finding for rats fed beef fat (4).

Table II summarizes histological findings in the animals dying spontaneously in the later series. Pathology of thyroid and pituitary will be reported later. The main pathological findings were cardiac fibrosis, acute and chronic pyelonephritis and interstitial nephritis and pneumonia with abscess formation. Such findings are usually noted in old rats. The severity of the lesions was graded from ± to ++++ and only lesions of at least ++ severity are included in the Table. No definite differences can be discerned among the groups. In the earlier study, there was noticeably more scarring in the hearts of the rats fed oxidized cottonseed oil(1). There was a small but consistent decrease in tumor incidence among the rats fed the oxidized form of each fat as follows:

TABLE II. Incidence of Severe Organ Pathology in Rats Dying Spontaneously After Having Been Fed Various Fresh and Mildly Oxidized Fats in an Iodine-Low Diet. Thyroid and pituitary pathology has been excluded.

		No. in group	Heart	Kidney	Lungs
Butter	Fresh	19	14	9	6
	Oxidized	24	14	9	4
Lard	Fresh	23	13	9	5
	Oxidized	10	3	2	5
Corn	Fresh	12	6	6	4
	Oxidized	12	9	2	3
Soybean	Fresh	12	6	3	6
	Oxidized	9	6	5	5

	Butter	Lard	Corn oil	Soybean oil	Total
Fresh	7/15	7/18	3/6	3/7	20/46
Oxidized	3/20	1/5	1/7	0/8	5/40

The combined data gave a chi-square with Yates' correction(5) of 8.44, (P less than .01). This is of interest in view of the con-

tinuing discussion as to possible carcinogenic effects of oxidized fats(6).

Discussion. Michelsen and Yang(7) have recently reviewed the evidence that deleterious effects of feeding raw soybeans can be prevented by heating of the beans. Our data suggest that some of the toxic material(s) may occur in the oil and is not completely removed during the processing of the oil for human consumption.

The absence of changes in peroxide numbers despite 40 hours of aeration demonstrates the presence of considerable amounts of antioxidants which were not removed during processing, and one may speculate that alteration of these substances may account for the beneficial effect of the heating and aeration of vegetable oils since toxic antioxidants are known to occur in cottonseed oil (gossypol). By the same token, oxidation of the animal fats did not result in a longer life span because their anti-oxidant system consists essentially of tocopherols, which are not toxic.

Studies in which the survival rates of rats fed fresh oils have been compared with those of rats fed heated oils have been reported by Alfin-Slater *et al* (soybean oil, 8) and by Rice *et al* (cottonseed, corn, and hydrogenated vegetable oils, 9). Although there were no significant differences in either instance, in the study of Rice *et al* there was a slight increase in the survival rate of rats fed the heated corn oil and the heated hydrogenated vegetable oil; moreover, feeding of fresh hydrogenated vegetable oil also brought about a longer life span. However, in both these studies, heating conditions were so different from ours that a strict comparison cannot be made.

Summary. Feeding of mildly heated and aerated butter and lard reduced the average life span (although not statistically significantly) of male rats fed these fats at a level of 20% in their diets. With similarly treated soybean and corn oils, survival was as good as with fresh animal fats whereas feeding of fresh vegetable oils led to significantly shorter life spans. The results were in agreement with those of a previous study comparing beef and chicken fats with olive oil. Thus, fresh olive oil, soybean oil, and corn oil appeared to contain, despite processing for human consumption, materials toxic to rats. These substances were inactivated by mild heating and aeration.

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