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Effect of dietary saturated and unsaturated oils on the longevity of laboratory animals

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THE EFFECT OF DIETARY SATURATED AND UNSATURATED OILS ON THE LONGEVITY OF LABORATORY ANIMALS

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Introduction

With the advent of the antibiotic era, attention has turned from the infectious diseases to the degenerative diseases and the ageing process in general as the major field of endeavor of those who would lengthen the human lifespan. Figures from the National Office of Vital Statistics (1) show that among the degenerative diseases, those of the cardiovascular system account for over half of all deaths, and that ischemic heart disease is the leading cause of death in persons over the age of 35 years.

Atherosclerosis is generally accepted as the predominent pathologic entity underlying ischemic heart disease (2), although other factors such as alterations in blood coagulability, decreased fibrinolysis, and changes in lipid clearing and capillary fragility have been proposed. With research into the etiology and mechanism of ischemic heart disease, there have been numerous factors postulated to influence the process. Among these are stress, physical activity, tobacco, climate, tissue trauma, and diet, as well as hereditary and genetic factors. Of these, diet and alterations in metabolism seem to offer the most promise and currently occupy the attention of the majority of workers. Their avenues of approach to the problem include such considerations as vitamin deficiency and deficiency of essential fatty acids, excessive and inadequate total caloric intake with the related problem of obesity, alterations in the protein and carbohydrate intake, and finally and perhaps most significantly, alterations in lipid intake and metabolism. The lipid problem may be further divided into considerations of cholesterol metabolism and the significance of serum and dietary cholesterol levels, total fat intake, and the quality of the fat in the diet with regard to chemical saturation or unsaturation. Excellent detailed considerations of the above observa-tions may be found in summary articles by Ahrens (3) and Jolliffe (4).

The saturated versus unsaturated fat problem has received widespread publicity recently and because of the promotion of commercial interests, there is currently a dietary fad of consuming increased amounts of unsaturated fats at the expense of saturated ones. That significant benefits may be obtained from the increased intake of unsaturated fats without adverse side effects has not been definitely established. Indeed, it has been proposed on theoretical grounds that the prolonged intake of unsaturated fats may be detrimental to health by accelerating the atherosclerotic process (5). The difficulties involved in performing long term experiments with rigidly controlled dietary intake, activity, and living conditions on a human population are obvious. In view of the above considerations and realizing the problems inherent in applying animal research to human populations, the experiment which will be reported in this thesis was devised by Denham Harman M.D., Associate Professor of Biochemistry and Associate in Internal Medicine at the University of Nebraska College of Medicine.

Method and Materials

The experimental animals consisted of 86 C3H female mice, 77 Swiss male mice, and 38 Sprague-Dawley male rats, all supplied by Jackson Memorial Laboratories, Bar Harbor, Maine. The mice were housed in plastic cages with perforated metal covers, five to ten mice per cage, on San-I-Cel bedding supplied by Laurel Farms and Paxton Processing Company of Paxton, Illinois. The cages were supplied with individual feeding dishes and water bottles, and were kept in racks in an animal room maintained at a temperature of about 80 degrees Farenheit. The cages were cleaned at two day intervals, and the feeding dishes and water bottles at weekly intervals or as necessary. All equipment was washed with water and dipped in a disinfectant solution of Hytron, active ingredient para di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium cloride monohydrate, supplied by Keystone Plastics Company, Media, Pennsylvania. The rats were housed in metal cages with wire mesh floors over metal pans to facilitate handling of excreta. Two animals were housed in each cage, each of which was also provided with individual feeding and watering containers. The cages were cleaned two to three times per week, and the food dishes and water bottles were handled in the same fashion as described for the mouse equipment. The cages were maintained in a rack in an animal room similar to that in which the mice were housed.

The feeds used were Rockland Mouse Diet and Rockland Rat Diet, supplied by the A. E. Staley Company of Decatur, Illinois. The composition of the diets is the same, however the mouse diet is milled somewhat finer than the rat diet. It consists of soybean oil meal, fish meal, dried whole milk, irradiated brewers type yeast, ground oats, wheat standard middlings, ground yellow corn, ground barley, feeding oat meal, ground whole wheat, dehydrated alfalfa meal, 1% animal fat, vitamin A palmitate, ½% steamed bone meal, 1% calcium carbonate, and 1% salt. The diet is stated by the manufacturer to "contain all the known nutrients needed for good nutrition. No other supplement need be fed . . .". Average analysis includes protein 24.27%, fat 4.15%, and carbohydrates 56.23%. Complete analysis and assays including vitamin, mineral, and amino acid content are available from the manufacturer (6). This diet was fed to the control animals without further alterations or additions. The animals fed on a high saturated fat diet were given the basic feed to which was added 10% by weight of olive oil supplied as Paragon Olive Oil by Magnus, Mabee, and Reynard Inc. of New York

City. A similar diet high in unsaturated fat was pre-pared by adding 10% by weight of corn oil, supplied as Mazola Corn Oil by the Corn Products Refining Company of Argo, Illinois to the basic diet. These diets represent an increase in calories derived from fat from 14.7% in the stock diet to 37% in the mixtures. The animals were fed daily, ad lib, in the following groups: C3H mice -34 control, 26 olive oil, and 26 corn oil fed animals; Swiss mice - 39 olive oil and 38 corn oil fed animals; Sprague-Dawley rats - 18 olive oil and 20 corn oil fed animals. The experimental diets were started in the mice at 5½ months of age and in the rats at 9 months of age, and were continued until the animals reached approximately 24 months of age, at which time the data set forth herein were compiled.

All animals were counted, weighed and examined for gross abnormality at monthly intervals, and all animals that died during the experiment were autopsied and specimens preserved for histologic examination. In the mice specimens were taken from the heart, liver, lung, kidney, and any tumors present. Similar specimens were taken in the rats as well as aorta, skin and skeletal muscle.

Results and Observations

The effect of high saturated and unsaturated fat diets on the longevity of the experimental animals is summarized in tables I, II, and III, on pages 13, 14, and 15. These tables represent a tabulation of all the mortality data obtained in the experiment, and the graphs and calculated values to be discussed in subsequent paragraphs are derived from this source.

Figure I, on page 16 is a graphic representation of percent survival versus age in months in the C3H mice. It will be noted that there is little difference in the curves representing the olive oil and corn oil fed mice. That both groups of animals fed a high fat diet did less well than those fed the control diet is equally obvious. The average longevity in months, of the control, olive oil, and corn oil fed animals respectively was 15.2, 12.0 and 11.3. The half survival time (age at which 50% of the animals were dead) for the control group was 14.0 months as compared to 10.2 and 9.8 months for the olive oil and corn oil fed groups. These figures bear out the impression given by the graph that the animals which received the high fat diets had a shortened survival time. The average longevity of the olive oil group was shortened by 3.2 months or 21%, and of the corn oil group by 3.9 months or

26% compared to the control group. The olive oil group outlived the corn oil group on an average of 0.7 months or by 6%.

Figure II, on page 17, depicts percent survival versus age in months in the Swiss mice. Again there is little to choose between the olive oil and corn oil fed groups, the olive oil group having survived only slightly longer than the corn oil group. The half survival time in the case of the olive oil fed mice was 15.5 months compared to 15.0 months for the corn oil fed animals. The upper curve in this graph represents data from a group of Swiss mice fed a control diet in a previous experiment, and is included here to provide a basis for evaluating the effect of the high fat diets on longevity, realizing that this is not a satisfactory substitute for a control group in this experiment, and that the validity of close statistical comparison of this group with the olive oil and corn oil groups is open to question. Nevertheless, the increased mortality of the high fat groups over the control group is obvious, and is consistent with the results of the C3H mouse experiment represented by figure I.

Figure III, on page 18, depicts percent survival versus age in months in the Sprague-Dawley rats. The curves again show that the olive oil fed animals outlived the corn oil fed group, and this is supported by the longevity data. The average longevity of the olive oil group was 21.2 months compared to 19.7 months for the corn oil group. The half survival time of the olive oil fed animals was 21.0 months compared to 19.0 months for the corn oil fed group.

Observations made on the C3H mice during the course of the experiment included, in the control group, four breast tumors and one corneal opacity. In the olive oil group, the coats of all the animals were noted to have an oily sheen, there were two breast tumors, one corneal opacity, and one case each of severe alopecia, conjunctivi-tis, and circling behavior. In the corn oil fed group, all animals were again noted to have oily coats, and there were two cases each of corneal opacity, alopecia and breast tumor. It should be noted that the C3H strain of mice have an apparently inbred predisposition to develop breast tumors, believed to be at least partially dependent on transmission of a viral agent through the mothers' milk (7). These tumors reach a size of two to three centimeters with caseation, necrosis, and hemorrhage at the center.

Observations made on the Swiss mice during the course of the experiment included, in the olive oil fed animals, conjunctivitis in three, corneal opacity in six, one ear tumor, two tumors of the abdominal wall, and one case of severe alopecia. In the corn oil fed animals there were seven corneal opacities, five cases of conjunctivitis, one of alopecia, one animal exhibited circling behavior, and one animal became paraplegic.

Gross examination of the autopsy specimens in the mice contributed no new information and histological preparations have not been made.

Observations made on the rats during the experiment included, in the olive oil group, four corneal opacities, and one animal with tic-like behavior, the spasm involv-ing rotation and extension of the neck. In the corn oil group there was one case of conjunctivitis, and four corneal opacities.

Autopsy findings in the rats included pneumonia in six olive oil fed animals, and three corn oil fed animals, lung abscesses in four of the olive oil group and one of the corn oil group, and one case of volvulus in each group. Hemotoxylin and eosin preparations of the tissues bore out the gross findings, with some degree of pneumonia being present in most animals. Stains for lipid material in the tissues have not yet been completed. This is thought to be appropriate because in numerous lung specimens, there were miliary deposits of lipoid appearing material. These may represent only miliary abscesses however.

Table I. Effect of High Fat Diets Containing Olive Oil or Corn Oil on Mortality Rates and Weights of C3H Mice.

Age Months	C3H Mice - Female								
	Diet								
	Control			Olive Oil			Corn Oil		
	No.L*	Wt.+	% L‡	No.L	Wt.	% L	No. L	Wt.	% L
5.0	34	-	100	26	-	100	26	-	100
5.5ǂ	34	-	100	26	-	100	26	-	100
6.0	-	-	-	-	-	-	-	-	-
6.5	34	-	100	23	-	88.5	23	-	88.5
7.0	32	20.9	94.2	21	18.4	81.0	22	19.9	84.6
7.5	-	-	-	-	-	-	-	-	-
8.0	-	-	-	-	-	-	-	-	-
8.5	30	22.2	88.3	17	21.5	65.5	19	19.7	73.0
9.0	30	22.6	88.3	17	21.6	65.5	17	20.2	65.5
9.5	-	-	-	-	-	-	-	-	-
10.0	-	-	-	-	-	-	-	-	-
10.5	28	23.6	82.5	12	21.4	46.2	11	19.6	42.3
11.0	-	-	-	-	-	-	-	-	-
11.5	25	-	73.5	12	-	46.2	11	-	42.3
12.0	24	23.1	70.6	11	21.8	42.3	10	20.7	38.5
12.5	-	-	-	10	-	38.5	10	-	38.5
13.0	22	23.1	64.6	10	22.0	38.5	10	22.3	38.5
13.5	-	-	-	-	-	-	-	-	-
14.0	17	26.8	50.0	10	21.1	38.5	10	23.4	38.5
14.5	-	-	-	-	-	-	-	-	-
15.0	17	24.5	50.0	7	22.1	27.0	9	21.0	34.6
15.5	-	-	-	-	-	-	-	-	-
16.0	13	24.3	38.2	5	22.2	19.4	5	22.0	19.4
16.5	-	-	-	-	-	-	-	-	-
17.0	11	23.7	32.3	5	19.8	19.4	3	22.0	11.5
17.5	-	-	-	-	-	-	-	-	-
18.0	11	24.0	32.3	5	21.1	19.4	2	21.0	7.7
18.5	-	-	-	-	-	-	-	-	-
19.0	11	23.3	32.3	2	19.5	7.7	2	22.5	7.7
19.5	-	-	-	-	-	-	-	-	-
20.0	6	25.1	17.6	2	21.5	7.7	1	20.0	3.8
20.5	-	-	-	-	-	-	-	-	-
21.0	5	17.4	14.7	2	22.0	7.7	1	21.0	3.8
21.5	-	-	-	-	-	-	-	-	-
22.0	5	22.0	15.7	1	16.0	3.8	1	21.0	3.8
22.5	-	-	-	-	-	-	-	-	-
23.0	4	24.0	11.8	1	18.0	3.8	1	21.0	3.8
23.5									
24.0									

Key: * Number of animals alive. + Average weight in grams.
‡ Percent of original group still living.
ǂ Fat diets started.

Table II. Effect of High Fat Diets Containing Olive Oil or Corn Oil on Mortality Rates and Weights of Swiss Mice.

Age Months	Swiss Mice - Male								
	Diet								
	Control**			Olive Oil			Corn Oil		
	No.L*	Wt.+	%L‡	No.L	Wt.	% L	No.L	Wt.	% L
5.0	50	27.2	98.0	39	-	100	38	-	100
5.5ǂ	-	-	-	39	-	100	38	-	100
6.0	48	29.0	94.0	39	-	100	38	-	100
6.5	-	-	-	-	-	-	36	-	94.7
7.0	48	29.6	94.0	37	-	94.6	-	-	-
7.5	-	-	-	34	36.9	87.0	35	34.7	92.2
8.0	48	31.0	94.0	-	-	-	-	-	-
8.5	-	-	-	-	-	-	-	-	-
9.0	47	32.0	92.4	32	37.0	82.0	30	37.3	79.0
9.5	-	-	-	32	36.3	82.0	30	36.9	79.0
10.0	47	32.8	99.2	-	-	-	-	-	-
10.5	-	-	-	-	-	-	-	-	-
11.0	46	32.6	90.4	26	39.8	66.5	30	36.7	79.0
11.5	-	-	-	-	-	-	-	-	-
12.0	46	32.6	90.2	26	-	66.5	29	-	76.2
12.5	-	-	-	26	38.9	66.5	27	37.0	71.0
13.0	46	33.0	90.2	-	-	-	-	-	-
13.5	-	-	-	23	38.7	59.0	25	37.1	65.6
14.0	38	33.0	74.5	-	-	-	-	-	-
14.5	-	-	-	21	39.2	53.8	20	37.5	52.5
15.0	37	32.0	72.5	-	-	-	-	-	-
15.5	-	-	-	20	37.6	51.0	17	35.9	44.6
16.0	37	32.0	72.5	-	-	-	-	-	-
16.5	-	-	-	16	38.7	41.0	12	33.5	31.5
17.0	36	35.0	70.6	-	-	-	-	-	-
17.5	-	-	-	12	38.8	30.8	9	35.1	23.7
18.0	35	35.2	68.6	-	-	-	-	-	-
18.5	-	-	-	9	38.1	23.0	7	31.2	18.4
19.0	32	35.0	62.6	-	-	-	-	-	-
19.5	-	-	-	7	36.6	17.8	3	36.0	7.9
20.0	30	34.8	58.8	-	-	-	-	-	-
20.5	-	-	-	7	33.6	17.8	3	34.7	7.9
21.0	29	33.6	57.0	-	-	-	-	-	-
21.5	-	-	-	4	40.1	10.3	3	34.2	7.9
22.0	-	-	-	-	-	-	-	-	-
22.5	-	-	-	4	38.6	10.3	2	37.0	5.3
23.0	-	-	-	-	-	-	-	-	-
23.5	-	-	37.3	2	35.5	5.1	2	36.0	5.3
24.0	19	34.2							

Key: * Number of animals alive. + Average weight in grams.
‡ Percent of original group still living.
ǂ Fat diets started. ** Data from a previous experiment -- the mice were on a pelleted diet.

Table III. Effect of High Fat Diets Containing Olive Oil or Corn Oil on Mortality Rates and Weights of Sprague-Dawley Rats.

Age Months	Sprague-Dawley Rats - Male					
	Diet					
	Olive Oil			Corn Oil		
	No. L*	Wt.+	%L‡	No. L	Wt.	%L
5.0	18	-	100	20	-	100
5.5	-	-	-	-	-	-
6.0	18	-	100	20	-	100
6.5	-	-	-	-	-	-
7.0	18	-	100	20	-	100
7.5	-	-	-	-	-	-
8.0	18	-	100	20	-	100
8.5	-	-	-	-	-	-
9.0‡	18	-	100	20	-	100
9.5	-	-	-	-	-	-
10.0	18	515	100	20	508	100
10.5	-	-	-	-	-	-
11.0	18	537	100	20	532	100
11.5	-	-	-	-	-	-
12.0	18	550	100	20	547	100
12.5	-	-	-	-	-	-
13.0	18	551	100	20	542	100
13.5	-	-	-	-	-	-
14.0	18	556	100	20	563	100
14.5	-	-	-	-	-	-
15.0	18	558	100	20	561	100
15.5	-	-	-	-	-	-
16.0	-	-	-	-	-	-
16.5	17	495	94.5	17	547	95
17.0	-	-	-	-	-	-
17.5	-	-	-	-	-	-
18.0	13	471	72.2	13	452	65
18.5	-	-	-	-	-	-
19.0	13	496	72.2	10	517	50
19.5	-	-	-	-	-	-
20.0	10	487	55.5	9	493	45
20.5	-	-	-	-	-	-
21.0	9	496	50.0	7	475	35
21.5	-	-	-	-	-	-
22.0	9	424	50.0	4	450	20
22.5	-	-	-	-	-	-
23.0	8	389	44.5	3	362	15
23.5	-	-	-	-	-	-
24.0	3	432	16.7	1	424	5

Key: *Number of animals alive. +Average weight in grams. ‡ Percent of original group still living. ‡Fat diets started.

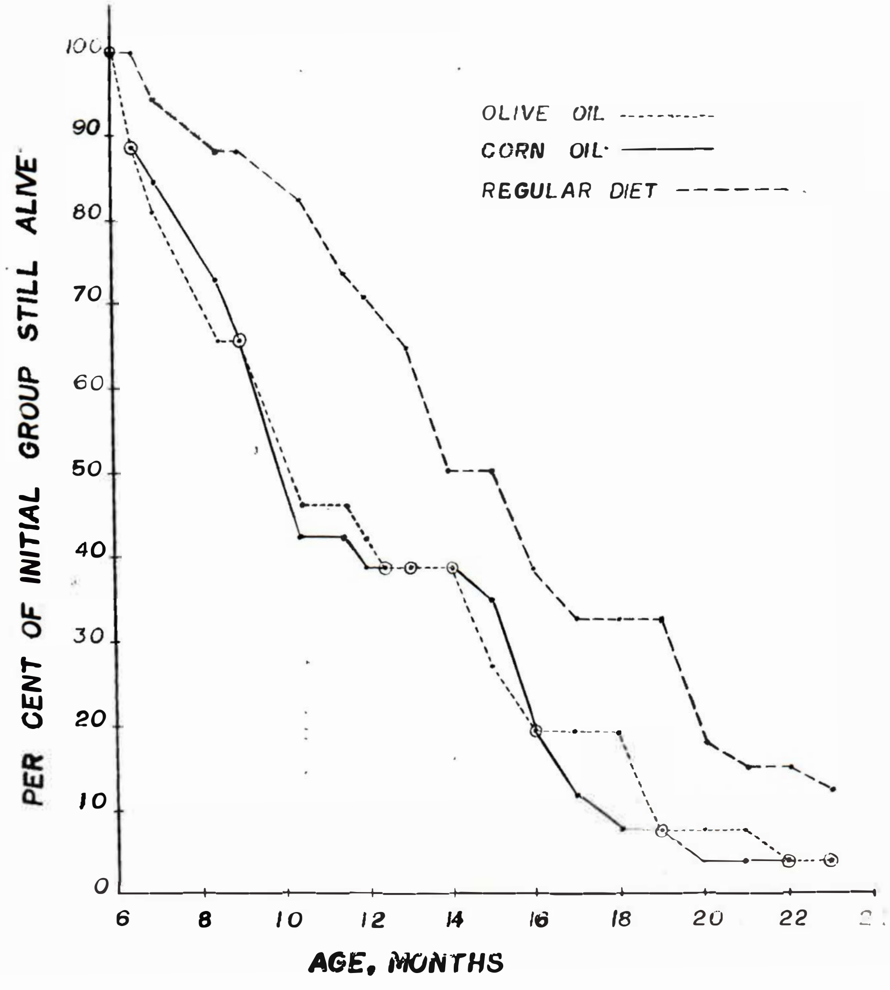

Figure I. Percent Survival Versus Age In C3H Mice Fed High Fat Diets Containing Olive Oil and Corn Oil.

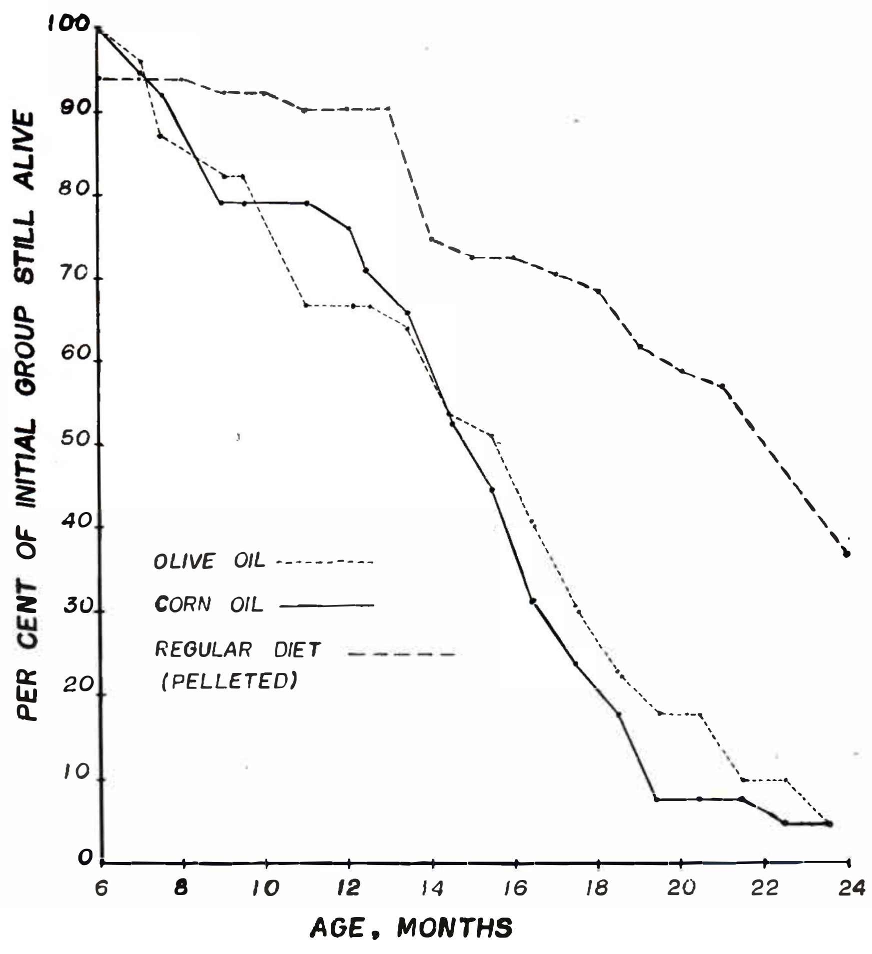

Figure II. Percent Survival Versus Age In Swiss Mice Fed High Fat Diets Containing Olive Oil and Corn Oil.

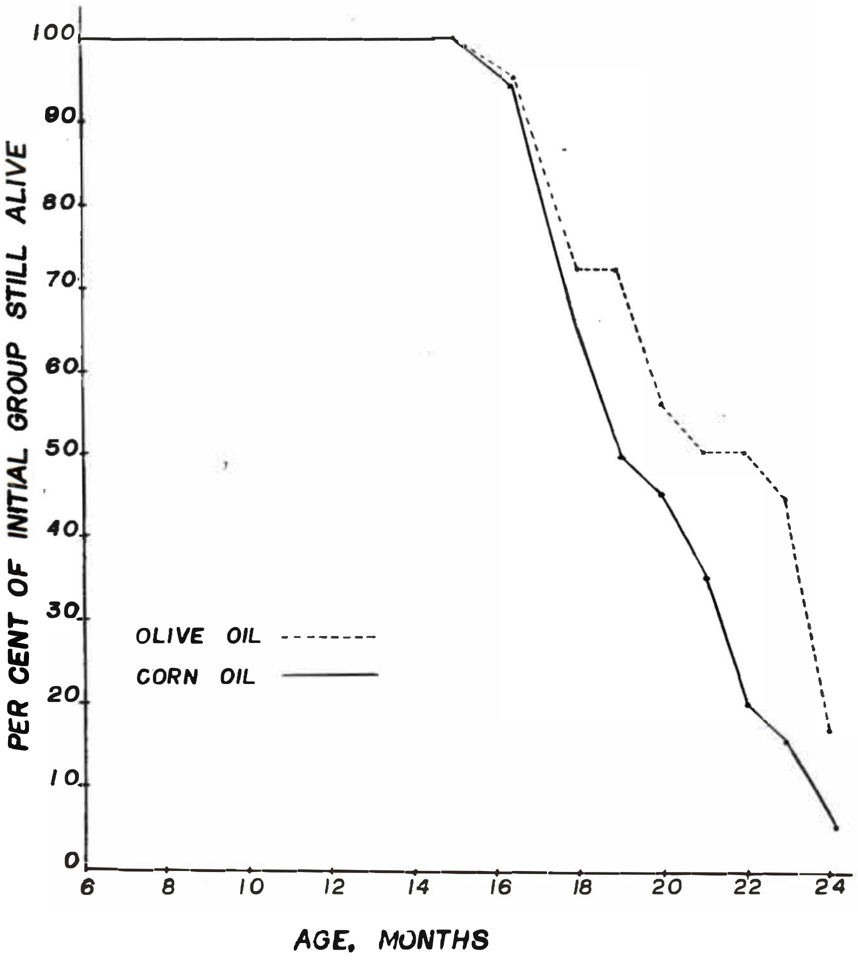

Figure III. Percent Survival Versus Age in Sprague-Dawley Rats Fed High Fat Diets Containing Olive Oil and Corn Oil.

Discussion and Conclusions

Interest in the significance of the chemical saturation of dietary lipids goes back over 20 years to the work of Snapper in 1941 (8). He studied the iodine numbers of serum lipids and theorized that the absence of various diseases in certain populations, among them arteriosclerosis, might be related to a high dietary intake of linoleic acid, an unsaturated fatty acid. It was not until 1952 however, that Kinsell (9) showed that the ingestion of certain unsaturated vegetable oils produced a lowering of serum cholesterol and phospholipid levels, thus suggesting a relationship between unsaturated fats and atherosclerosis. This finding has since been verified by numerous workers including Bronte-Stewart et al (10) who showed that the serum cholesterol lowering effect of marine and vegetable oils was destroyed by hydrogenation. Much interest has thus been aroused and there are numerous theories dealing with the way in which unsaturated fats exert their effect on serum cholesterol levels, including considerations of the degree of unsaturation, essential fatty acid content, and the ratio of unsaturated to saturated fats (4). The significance of an elevated serum cholesterol level in the atherosclerotic process and ischemic heart disease is supported by population studies relating an increase in serum cholesterol to ischemic heart disease (11, 12), tissue culture studies where addition of cholesterol to the media produces an increase in cholesterol deposition in human aortic intimal cells (13), and on work with experimental animals where an increase in atheromata is correlated with high serum cholesterol levels and can be influenced by feeding unsaturated fats (14). Although the mechanism of this effect remains unknown, numerous dietary regimens embodying the unsaturated fat concept are now being promoted by workers in the field (15, 16, 17), as well as by commercial interests. Before recommending such diets however, one might ask whether large populations can be induced to observe dietary restrictions the merits of which remain unproven, and even more importantly, will such diets significantly reduce the death rate from ischemic heart disease, and what will be the long term side effects of an unsaturated fat diet? In considering this problem, Harman in 1957 (5) pointed to an increased incidence of gastric carcinoma in populations subsisting on a diet high in unsaturated fat, and to similar findings in experimental animals. He also proposed that since unsaturated fats are readily oxidized, they may have an adverse effect on certain enzymes, and may even accelerate the atherosclerotic process on the theoretical grounds that one of the early steps in atherosclerosis may be an oxidative polymerization of lipoproteins. He points out that in the oxidation of unsaturated fats free radicals are formed, e.g. peroxy and hydroxy radicals, identical to those produced by the irradiation of tissue, and possibly related to the carcinogenic property of radiation. These same free radicals have been proposed to be active participants in the ageing process (18), and hence one might expect that the increased production of these entities would accelerate ageing.

Although gastric carcinoma and atherosclerosis were not demonstrated in this experiment, the results are consistent with the theory of accelerated ageing proposed above, in that in all three groups of animals, those fed an unsaturated fat diet did not live as long as those fed the saturated fat diet. While comparison of saturated versus unsaturated fat diets is possible from the data obtained, the overall picture is clouded by the fact that neither group of animals on the high fat diets lived as long as the control animals. This observation, however, is consistent with the work of Silberberg and Silberberg in 1954 (19) who fed a diet of 29% fat to mice and observed a decrease in longevity of from 9 to 16%, and of French et al in 1953 (20) who produced a similar decrease in longevity in rats by feeding a fat supplemented diet. In the first case, the diet contained lard, a saturated fat, and in the second case it contained corn oil, an unsaturated fat. Furthermore, the human population studies of Keys and White (21) showed that groups with 40% of total calories derived from fat had high death rates as compared to groups where 20% or less of the total calories were derived from fat. This type of study has been much maligned however on the grounds that it does not take into consideration the degree of saturation of the dietary fat. Jolliffe and Archer (22) believe that this alone will explain the discrepancies arising among the various studies, however Yudkin in 1957 (23) made an extensive study which showed that total calories in the diet correlated more closely with heart disease than did the number of fat calories or the number of calories derived from animal fat and that vegetable fats and hydrogenated fats alone did not correlate any more closely than did total fat calories. He also observed however, a threshold of about 30% fat calories above which heart disease was common. Due to the difficulty of interpreting the results of human population studies, it would seem wise to continue this work using animals, where conditions are more readily controlled and the results are more easily interpreted.

It is therefore concluded that in the study reported here, the effect of the degree of saturation of the dietary supplement is minimal, and although it is slightly in favor of the saturated compound, it is greatly overshadowed by the detrimental effect of the increase in total fat colories, both saturated and unsaturated. Accordingly, recommendation of limitation of the total fat intake would seem more prudent than the use of unsaturated fats in the diet.

The observations of pathology in these animals did not follow any numerically significant trends, with the exception of the large number of chronic pulmonary infections observed in the rats, a finding similar to that reported by Berg and Harminson in 1957 (24). This was doubtless the chief cause of death among the rats, while among the mice, no outstanding cause of death was apparent.

In designing future experiments, adequate controls should be provided for each group of experimental animals, and the saturated and unsaturated fat diets should be fed in eucaloric amounts to observe the effects of the degree of saturation as well as in the manner of this experiment to study the influence of total fat intake. Experiments of this type, in spite of species differences, should provide significant information which will lead to an eventual understanding of atherosclerosis and ischemic heart disease, their cause and prevention.

Summary

Diseases of the cardiovascular system cause over half of all deaths, and ischemic heart disease is the leading cause of death of people over 35 years of age. Atherosclerosis is considered to be the predominent pathologic entity in ischemic heart disease and has been attacked by studies involving lipid intake and metabolism. Unsaturated fats have been shown to lower serum cholesterol and phospholipid levels, and recently have been proposed as a dietary means of decreasing atherosclerosis and prolonging life, although their effectiveness is unproven and the nature of their long term side effects is unknown. In a two year study, two species of mice and one species of rat were fed diets supplemented with unsaturated and saturated fats to a level of 37% fat calories. In all three groups of animals, those fed the saturated fat diet lived slightly longer than those receiving the unsaturated fat diet. The survival times of both groups, however, were markedly shorter than the survival time of the animals fed a control diet. Literature references suggesting a possible explanation of the experimental observations are cited. It is concluded that in this experiment, the high total fat intake had a more detrimental effect on longevity than did the degree of saturation of the dietary fats. Limitation of total fat intake

rather than use of unsaturated fats would seem a prudent recommendation to those who would decrease atherosclerosis and live longer.

Bibliography

1. United States Department of Health, Education, and Welfare, Public Health Service, National Office of Vital Statistics, Vital Statistics of the United States 1:6-18, 6-23, 1958.

2. Katz, L. M., Stamler, J., and Pick, R., Research Approach to Atherosclerosis, J.A.M.A. 161:536, 1956.

3. Ahrens, E. H. Jr., Nutritional Factors and Serum Lipid Levels, Am. J. Med. 23:928, 1957.

4. Jolliffe, N., Fats, Cholesterol, and Coronary Heart Disease, Circulation 20:109, 1959.

5. Harman, D., Possible Ill Effects of the Use of Highly Unsaturated Fats to Lower Serum Cholesterol Levels, Lancet 2:1116, 1957.

6. A. E. Staley Manufacturing Company, Decatur, Illinois, Rockland Laboratory Animal Stock Diets.

7. Bittner, J. J., Experimental Aspects of Mammary Cancer in Mice. Breast Cancer and Its Diagnosis and Treatment. (Lewison, E. F., ed.). Baltimore, Williams and Wilkins, 1955, pp. 75-102.

8. Snapper, I., Chinese Lessons to Western Medicine. p. 30, New York, 1941. Interscience Publishers Inc.

9. Kinsell, L. W., et al, Dietary Modification of Serum Cholesterol and Phospholipid Levels, J. Clin. Endocrinol. 12:909, 1952.

10. Bronte-Stewart, B., et al, Effects of Feeding Different Fats on Serum Cholesterol Level, Lancet 1:521, 1956.

11. Gofman, J. W., et al, Evaluation of Serum Lipoprotein and Cholesterol Measurements as Predictors of Clinical Complications of Atherosclerosis, Circulation 14:691, 1956.

12. Keys, A., Diet and the Development of Coronary Heart Disease, J. Chron. Dis. 4:364, 1956.

13. Rutstein, D. D., et al, Effects of Linoleic and Stearic Acids on Cholesterol Induced Lipoid Deposition in Human Aortic Cells in Tissue Culture, Lancet 1:545, 1958.

14. Malmros, H., and Wigand, G., Atherosclerosis and Deficiency of Essential Fatty Acids, Lancet 2: 749, 1959.

15. Central Committee for Medical and Community Program of the American Heart Association, Dietary Fat and Its Relation to Heart Attacks and Strokes. J. Amer. Med. Assn. 175/5:389, 1961.

16. Pilkington, T. R. E., et al, Practical diets for Lowering Serum Lipids; A Long Term Study on Out-patients With Ischemic Heart Disease, Brit. M. J. 1:23, 1960.

17. Proger, S., Current Status of Diet and Lipid Metabolism in the Pathogenesis of Atherosclerosis, Progr. Cardiovasc. Dis. 1:134, 1958.

18. Harman, D., Mutation, Cancer, and Ageing, Lancet, 1: 200, 1961.

19. Silberberg, M. and Silberberg, R., Factors Modifying the Lifespan of Mice, Am. J. Physiol. 177:23, 1954.

20. French, C. E. et al, Influence of Dietary Fat and Carbohydrate on Growth and Longevity in Rats, J. Nutrition 51:329, 1953.

21. Keys, A. and White, P. D., World Trends in Cardiology. Vol. I, Cardiovascular Epidemiology. New York, Paul B. Hoeber Inc. 1956.

22. Jolliffe, N., and Archer, M., Statistical Associations Between International Coronary Heart Disease Death Rates and Certain Environmental Factors, J. Chron. Dis. 9:636, 1959.

23. Yudkin, J., Diet and Coronary Thrombosis, Hypothesis and Fact, Lancet 2:155, 1957.

24. Berg, B., and Harminson, C., Growth, Disease, and Ageing in the Rat, J. Gerontol. 12:370, 1957.

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