Alteration of Serum Cholesterol by Dietary Fats.* (23461)

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The concentration of cholesterol in the blood serum of man can be reduced through subsistence on a low-fat diet(1-5) or by substitution of certain vegetable oils in the diet (6-11). Recognition of the fact that an elevated serum cholesterol level tends to be related to the development of atherosclerosis has led to widespread interest in the possibility that regulation of the fat content of the diet may help to control or prevent ischemic (coronary) heart disease(4,8,12,13). One result of this attitude is the recent use of corn oil in the diet, sometimes on medical prescription, by persons with hypercholesterolemia or coronary heart disease or both.

Corn oil when fed in large amounts has been repeatedly shown to produce a large depression in the serum cholesterol level(8-11, 13,14). This effect has been attributed to its high content of polyunsaturated fatty acids by some workers(8,9,13,14), but the possibility that this characteristic of corn oil explains only part of the effect is also indicated(11,13, 15,16). Examination of these reports shows that in some cases the subjects had metabolic peculiarities including hypercholesterolemia, obesity and diabetes. In many of the studies synthetic and artificial mixtures instead of natural foods were used as diets, and in practically all cases the experimental fat was isocalorically substituted for carbohydrate or for another fat. Further, in many of the studies the numbers of persons studied did not permit proper statistical analysis.

Accordingly, it was considered desirable to compare several food fats, including corn oil, in regard to the influence on the serum cholesterol, when taken as a simple supplement to the diet in the manner now being used by an appreciable number of patients, and to make such a comparison with enough subjects, relatively homogeneous in type, to allow valid statistical analysis. *Subjects.* The subjects were the 141 volunteers in the laboratory courses in Physiological Chemistry who satisfactorily completed the prescribed dietary regimen for which 159 originally volunteered. Their mean age was 22.8 years (range 20-34). Two of the volunteers were young staff members, 10 were graduate students, 16 were Medical Technology students and 113 were freshmen in the Medical School. These volunteers were organized by the class officers.

Materials and methods. The characteristics and source of the 4 fats employed are given in Table I. In order to make the dietary fat supplements palatable and to disguise their identity the fats were incorporated in "milkshakes." The composition of each milkshake was: fat 57 g (2 oz), fat-free dried milk 30 g, sucrose 10 g, Dariloid K† 0.5 g, a mixture of mono- and distearates of glycerol 0.5 g‡, double strength vanilla extract 1.0 ml, and water to make 300 g. The milkshakes containing the fat of cream each contained 171 ml of 35% whipping cream and were made with 20 g of dried fat-free milk instead of 30 g owing to the milk proteins contained in cream. The taste of the olive oil containing milkshakes was difficult to render acceptable and these were additionally flavored with coffee extract.

Each type of milkshake was prepared by homogenization in bulk quantity, pasteurized and packaged in 300 g amounts in color-coded individual paraffined paper milk cartons by the Dairy Husbandry Dept. of the Univ. of Minnesota. The preparations were kept

* The members of the Class of 1957 in Physiological Chemistry, Medical School, University of Minnesota, were the subjects. These students actively participated in planning of the work and in chemical and statistical analyses which were also independently carried out by the authors.

† A preparation of alginic acids used as an agent for stabilizing emulsions. Obtained from Kelco Co., Chicago, Ill.

‡ An emulsifying agent labeled Atmos 150. Obtained from Atlas Powder Co., Wilmington, Del.

TABLE I. Characteristics of Fats Tested.

Fat	Iodine No.	Linoleic acid, g/100 g fat	Linolenic acid, g/100 g fat
Cream	31.9	1.53	.60
Corn oil	126.7	57.5	.86
Safflower seed oil	144.2	72.0	.48
Olive oil	85.8	9.5	—

These analyses were kindly carried out by Dr. R. T. Holman, Hormel Institute, Austin, Minn. Cream used as 35% whipping cream; corn oil from Clinton Foods, Inc., Clinton, Iowa; edible safflower seed oil from Pacific Vegetable Oil Co., San Francisco, Calif.; olive oil as Gerber's U.S.P. olive oil.

frozen until the day before they were to be dispensed when they were transferred to a cold-room at 4°C.

Each student ingested one milkshake of his assigned code per day for 9 days and recorded on a chart in the laboratory the fact that he had or had not consumed the whole of each day's milkshake. The time-of-day and the rate at which the milkshakes were consumed were entirely self-chosen and varied considerably among the students. No attempt was made to regulate the basal diet of the students beyond indicating to them that they could reduce their consumption of bread and desserts if they were concerned about gain in body weight. The results of serum cholesterol determinations of all persons who failed on 2 or more days to consume a milkshake were excluded from the tabulation of the results. The data of 18 persons were excluded on this basis. Some of the individuals consuming the safflower oil containing supplements complained of diarrhea and dropped out of the study group for this reason. Fasting blood samples, from which the sera were harvested, were obtained by vena puncture just prior to ingestion of the first milkshake and on the day following the consumption of the last milkshake. Body weights were recorded on the same days. Each student made a single determination for total cholesterol content in his own 2 sera samples by the method of Kingsley and Schaffert(17). The analyses of the 2 samples were carried out concurrently, which permitted the colorimeter readings of the initial and final samples to be referred to the same developed cholesterol standard. The same sera were independently analyzed in duplicate for total cholesterol in the Laboratory of Physiological Hygiene by the method of Anderson and Keys(18).

No subject or other person who took part in the analyses or in the final compilation of the results was aware of the identity of the fat in the coded milkshakes until after the selection and summation of the results. The results of 4 analyses by students of initial and final serum samples were excluded because the recorded result departed from the mean initial result for the entire study group by more than 2 standard deviations. The initial and final analyses obtained by 11 other students were discarded because of known laboratory accidents or errors. The number of subjects given in the tables refers in each case to the number of individuals who satisfactorily completed the dietary regimen and on whose serum samples acceptable initial and final cholesterol determinations were obtained.

Results. The results are given in Tables II

TABLE II. Alteration of Serum Cholesterol Contents during Periods of Fat Intake.

Dietary group	No. of persons*	Initial serum cholesterol, mg/100 cc	Deviation of final serum cholesterol from initial value			
			mg/100 cc	%	t	P
Corn oil	A 34	226.7 ± 50.77†	−37.0 ± 6.16‡	−16.3	6.01	<.001
	B 32	205.4 ± 33.72	−23.0 ± 2.84	−11.2	8.10	<.001
Olive oil	A 35	234.0 ± 46.70	−14.2 ± 7.68	− 6.1	1.85	.07
	B 36	197.6 ± 27.11	−10.7 ± 2.32	− 5.4	4.62	<.001
Safflower oil	A 26	220.3 ± 32.84	−22.8 ± 5.92	− 9.9	3.85	<.001
	B 28	201.9 ± 34.55	−18.0 ± 4.56	− 8.9	3.95	<.001
Cream	A 31	228.6 ± 49.23	+ 8.9 ± 7.41	+ 3.9	1.2	.23
	B 32	194.6 ± 29.72	+ 2.8 ± 3.19	+ 1.5	.88	.37

* A = Data obtained by students. B = Data obtained by Laboratory of Physiological Hygiene.

† Mean ± S.D. ‡ Mean ± S.E.

TABLE III. Initial Body Weights and Body Weight Gains.

Dietary group	No. of persons	Initial body wt (mean ± S.D.)	Gain in body wt (mean ± S.E.)	Significance of body wt gains	
				t	P
Corn oil	32*	165.7 ± 25.25	1.28 ± .35	3.66	<.001
Olive oil	36	160.4 ± 20.47	.95 ± .37	2.59	.012
Safflower oil	28	164.8 ± 20.44	2.25 ± .37	6.10	<.001
Cream	32	155.0 ± 24.02	1.15 ± .32	3.60	<.001

* 2 subjects failed to record final wt.

and III. Statistical treatment of the results was made by a method of paired variates.§ This method gives a measure of the significance of the over-all differences between the initial and final observations for the individual subjects in each group.

The students obtained higher values for total serum cholesterol than were observed in the Laboratory of Physiological Hygiene on analysis of the same sera primarily, it is believed, because of differences in methodology in analytical procedures. The method used by the students does not involve hydrolysis of the cholesterol esters whereas the method of Anderson and Keys(18), a modification of the method of Abell *et al.*(19), effects hydrolysis with alkali before application of the Liebermann-Burchard reagent for development of the color. Since cholesterol esters give a higher color intensity than free cholesterol in the Liebermann-Burchard reaction and free cholesterol is used as the standard, a method which does not hydrolyze the esters will necessarily give high values. From Table II the magnitude of this systematic over-estimation in the students' analyses can be computed. In the 8 sets of comparisons (4 groups, each group "initial" and "final"), the students' averages range from 4.0 to 20.3% above those from the Laboratory of Physiological Hygiene, the grand mean discrepancy being 13.1%.

§ $t = \sqrt{\frac{(N-1)(\Sigma d)^2}{N\Sigma d^2 - (\Sigma d)^2}}$ where "N" is the number of cases and "d" is the difference between the initial and final observations for each subject. The calculated values of "t" are recorded in the tables in terms of probability that the results are due to chance. The calculations were independently checked by the authors.

The standard deviations of the means of the student analyses are larger than those of the mean results obtained by the Laboratory of Physiological Hygiene due, undoubtedly, to the lesser technical skill of the students. Nevertheless, owing to the large number of analyses, the same general conclusions, with one possible exception (olive oil group), are indicated by each set of determinations.

The groups ingesting the 3 vegetable oils each exhibited statistically significant decreases of total serum cholesterol while the subjects receiving cream exhibited a slight rise which is not significant statistically. The result obtained with the corn oil group was of greater magnitude than that obtained with the other 2 vegetable oils. The difference in effect produced by corn oil and olive oil appears to be real ($P<0.01$) but the data do not demonstrate a statistically significant difference between the effects produced by corn oil and safflower oil. Safflower oil is the most unsaturated and has the highest linoleic acid content of the oils tested (Table I), and if these characteristics were the sole determinants of the cholesterol depressing action of these vegetable oils, safflower oil should have exhibited a clearly superior effect over that of corn oil. Since this result was not obtained, and because the result was, in fact, in the opposite direction, it appears that the cholesterol lowering qualities of corn oil are not due solely to its content of linoleic acid or degree of unsaturation as expressed by the iodine value.

Each of the 4 groups of students exhibited significant increases in body weight over the 9 days of the experiment, the grand average gain being 1.37 lb or 0.62 kg. This fact indicates that the subjects did not reduce their caloric intake from other foods by an amount

equal to that of the milkshakes. Nevertheless, some reduction of intake of ordinary food of the diet must have occurred with a majority of the subjects because the weight gain, with the exception of the safflower oil group, was not as great as would have resulted had the milkshake calories been added to those of an unreduced pre-experimental diet. A gain of 1 kg from simple overeating represents tissues having a value of about 6180 calories(20). Since the caloric content of the milkshakes over the 9-day period was 6157 calories the subjects would all have gained about 1 kg had they not reduced their intake of ordinary food. Only in the case of the safflower oil group was the weight gain of this magnitude. The average weight gain of the other 3 groups was 1.1 lb or 0.5 kg. Hence it is indicated that these students stored on an average 3090 calories. Since the milkshakes provided 6157 cals. a total of 3067 cals., or 340 cals. per day, of ordinary food of the diet was supplanted by the milkshakes. It is not possible to determine what ordinary food items were involved in this dietary substitution but it must be assumed that a part of the reduction in the ordinary diet was at the expense of beef and dairy fats since these are prominent in the usual diet and are most easily omitted at the table. These considerations then lead to the point that a part of the reduction of serum cholesterol concentration in the subjects in the corn oil and olive oil groups may have been due to reduced intake of saturated fats. The same consideration also suggests that the cholesterol lowering in the safflower oil group might have been of a somewhat greater degree had the individuals in this group reduced their intake of ordinary food fat to the same extent as those of the other groups.

It would appear that the serum cholesterol changes observed may have been the result of the following factors: 1) addition to the diet of 57 g per day of the experimental fat, 2) removal of ordinary diet fat, mainly of the saturated type, 3) slight positive calorie balance with attendant requirement of extra fat transport in the body to fat stores, 4) operation of the factor associated with unsaturation of fatty acids in the experimenal fat, and 5) operation of the "X" factor in corn oil.

While evaluation of the contribution of each of the several factors listed above to the cholesterol change cannot be precise, the findings as to net effects of the several fats used as supplements show what may be expected with persons using such supplements with a view to serum cholesterol control. Corn oil is indicated to be the fat of preference but without any clear margin over safflower oil. Since the body weight response was almost identical in the groups ingesting butterfat, olive oil and corn oil, the results with these fats raise no question of full comparability as is the case with safflower oil.

Summary. Serum total cholesterol concentration was measured in 122 young men and 19 young women before and after 9 days during which each person ingested daily 57 g of corn oil, olive oil, safflower oil or butterfat. The subjects were instructed to follow their usual diets during this period and body weight measurements indicated that the experimental fats did not supplant an equal quantity of ordinary diet calories. The subjects who ingested butterfat showed a slight but statistically insignificant rise in the serum cholesterol while those who ingested the other fats exhibited a statistically significant decrease, averaging 23.0 $\pm$ 2.8 mg per 100 ml with corn oil, 18.0 $\pm$ 4.6 with safflower oil and 10.7 $\pm$ 2.3 with olive oil. Compared with the safflower oil the corn oil was more saturated (iodine value 126.7 vs. 144.2) and contained less linoleic acid (57.5 vs. 72.0%), so it is concluded that the cholesterol depressant action of the corn oil was not fully accounted for by its degree of unsaturation or content of "essential" fatty acid. Since the subjects in all groups gained some weight, it appears that at least some effects of the 3 vegetable oils tested can be obtained without exact isocalorie substitution in a normal diet.

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Procedure for Transplantation of Dog Kidney without Interruption of Renal Blood Supply.*† (23462)

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Kidneys transplanted from one dog into another are usually rejected within five to ten days(1). A characteristic pathology develops in these tissues and it is generally accepted that this rejection is mediated through an immunological reaction(2). The operative procedure previously employed for transplantation requires an interruption in the blood supply for at least 20 minutes. The contribution of the ischemic period to the development of the subsequent metabolic and pathologic states is unknown; however, it seems likely that it may produce non-specific damage to the renal tissues. We have devised a technic whereby the kidney can be transplanted without interruption of its blood supply, thus permitting study of the rejection process without the complicating factor of ischemia.

Methods. In principle, the new procedure takes advantage of the fact that the aorta and vena cava form a "T" connection with their respective renal vessels. This structural arrangement permits perfusion of the renal vessel from below during the time when the upper section of the aorta or vena cava must be occluded for the vascular suturing. For example, in the arterial system the blood may flow into the renal vessel either from above as it normally does or from below the point of origin of the artery on the aorta. In our procedure, the accessory circulation provides arterial blood for the kidney through the aorta via a connection through a plastic tube with the host's carotid artery. This blood enters the aorta below the point of origin of the renal vessel. The same principle is used to return the renal venous blood through the vena cava below the point of origin of the renal vein, and back through the external jugular to the donor's heart. The donor animal is prepared by dissection of the left kidney, its ureter and the renal vessels. The aorta and vena cava are also freed for a distance of 1½ inches above and 1½ inches below the renal vessels. All of the major abdominal vessels except those referred to above,

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