The Effect of Commercial Margarines on Plasma Cholesterol Levels in Man

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THE discovery that the substitution of certain vegetable oils for certain animal fats in the diet of man led to a decrease in serum cholesterol levels[1-3] and the demonstration that this phenomenon was caused by a hypercholesterolemic factor in the latter fats and a hypocholesterolemic one in the former[4,5] prompted an intensive search for these agents. In respect of the hypocholesterolemic agent in the corn oil, at least three possibilities were apparent immediately—tocopherols, plant sterols and unsaturated fatty acid residues were present in relatively large amounts in corn oil, the vegetable fat with which we worked initially. It was demonstrated early that alpha-tocopherol in amounts providing 30 to about 600 mg. per day had no effect on plasma cholesterol levels when added to a formula-type diet providing 60 per cent of calories in the form of butter fat.[6] Evidence was obtained, however, to indicate that the plant sterols accounted for a significant part at least of the hypocholesterolemic activity of corn oil.[6,7]

Our investigation of the effect of degree of unsaturation has led to results which differed according to the experimental conditions employed. Thus when subjects (university students) were stabilized on a formula ration in which the fat moiety was supplied in the form of 35 per cent of calories from butter fat and the basal ration modified by the addition of 25 per cent of calories as corn oil or hydrogenated corn oil (IV = 68) in place of an equicaloric amount of carbohydrate, the hypocholesterolemic response was the same for both preparations.[7] However, in a subsequent experiment[8] in which the subjects were stabilized on a fat-free diet, the addition of 45 per cent of calories as corn oil or hydrogenated corn oil (IV = 69) in place of an equicaloric amount of carbohydrate led to the usual significant decrease in plasma cholesterol in the case of the corn oil ration but no change in the case of the hydrogenated form. These experiments indicated that the effect of hydrogenated corn oil on plasma cholesterol depended upon the dietary conditions used. It had a significant hypocholesterolemic effect when added to a ration containing a hypercholesterolemic fat and no effect when added to a fat-free ration.

Despite these findings and other reports[9-12,16-18] indicating that degree of unsaturation of fats is not the sole or indeed even the most important determinant of hypocholesterolemic activity, the belief is widely held that the hydrogenation of vegetable oils as in the production of margarines renders them hypercholesterolemic. In view of the paucity of information on this point, we decided to test a number of commercially available margarines under two sets of conditions. Some of the work reported here has been presented in abstract form.[13]

EXPERIMENTAL

The experimental conditions and basal diet have been described in previous publications[5,6,14] and only a brief description will be given here. The volunteer subjects, comprised of university students and a few members of staff, ingested for a period of sixteen days a

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TABLE I

Composition of Basal Diet.* Amounts Required to Make a 950 Calorie Sample

Ingredient	Amount (gm.)	Protein (gm.)	Fat† (gm.)	CHO (gm.)
Mil-ko‡	28.1	10	0.1	14.6
Casec§	28.4	25	0.57	...
Sucrose	20.0	...	...	20.0
Dextrimaltose	169.8	...	...	166.4
Total	...	35.0	0.67	201.0
Calories	950.03	140.0	6.03	804.0
Per cent calories	...	14.74	0.63	84.63

* Two grams iodized salt were added per 950 calorie batch. A mixture of vitamins was also added to supply the following amounts of these substances per 950 calories: thiamine, 0.6 mg.; riboflavin, 0.6 mg.; niacin, 5.0 mg.; pyridoxine, 5.0 mg.; calcium pantothenate, 5.0 mg.; ascobic acid, 25 mg. 1,700 I.U. of vitamin A was added per 950 calorie portion.

† Fat was substituted equicalorically for the carbohydrate component, dextrimaltose.

‡ A brand of skim milk powder.

§ A brand of calcium caseinate.

basal formula ration. This was composed of the ingredients shown in Table I homogenized with water. Fat was added by equicaloric substitution for one of the carbohydrate components, dextrimaltose. The only other items permitted were water, tea and coffee without sugar or milk. A small number of subjects was unable to stay on the diet and dropped out for various reasons—illness or inability to adjust to the dietary regimen. Only a few gained or lost more than 2 or 3 pounds during the experimental period and the overwhelming majority were highly successful in keeping their body weight essentially constant. The average change in weight for experiments I and II between day 8 and day 16 was minus 0.8 pound and minus 0.4 pound, respectively. Blood samples were taken from the subjects in the fasting state between 7:00 and 8:30 A.M. on days 0, 4, 8, 12 and 16. Plasma cholesterol was determined by the technic of Abell et al.[15]

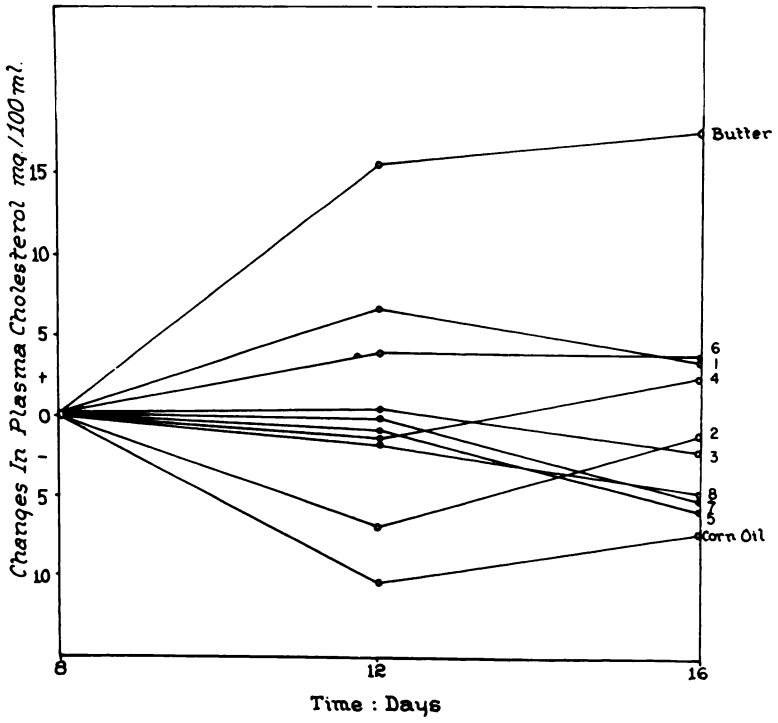

FIG. 1. Average changes in plasma cholesterol values in groups of university students from those observed after eight days on a diet providing 22.5 per cent of calories as butter following supplementation with an additional 22.5 per cent of calories as margarines, corn oil or butter, substituted at the expense of an equicaloric amount of carbohydrate (see Table II for numbers of subjects in each group).

TABLE II

The Effects on Plasma Cholesterol Levels of Substituting 22.5 per cent of Calories from Margarines Equicalorically for Carbohydrate in a Diet* Already Providing 22.5 per cent of Calories from Butterfat

Group No.	Type of Fat	No. of Subjects†	Mean Difference Between Day 8 and Day 16 (mg. cholesterol per 100 ml.)	Standard Error of Difference	t	P
1	Margarine	9	+3.6	5.69	0.63	0.6–0.5
2	Margarine	10	−1.2	4.38	0.28	0.8–0.7
3	Margarine	9	−2.3	3.42	0.67	0.6–0.5
4	Margarine	8	+2.4	5.79	0.41	0.7–0.6
5	Margarine	8	−5.8	2.78	2.07	0.1–0.05
6	Margarine	9	+3.6	4.21	0.85	0.5–0.4
7	Margarine	9	−5.4	6.41	0.85	0.5–0.4
8	Margarine	9	−5.2	3.70	1.39	0.3–0.2
9	Corn oil	8	−7.6	4.67	1.62	0.2–0.1
10	Butterfat	9	+17.3	2.39	7.25	<0.01

* All subjects had eaten the basal ration providing 22.5 per cent of calories as butterfat for a period of eight days.
† There were no females in group 5, one in groups 1, 3, 4, 6, 7, 9 and 10, and two in groups 2 and 8.

Eight commercial margarines were tested under the following conditions. In experiment I, ninety-five subjects (eighty-two men and thirteen women) were placed for eight days on a ration providing 22.5 per cent of calories from butter fat. The volunteer subjects were then divided into ten groups of comparable plasma cholesterol levels as determined by the values obtained at day 4. During the next eight days they consumed this diet modified by the addition of 22.5 per cent of calories from margarine at the expense of an equicaloric amount of carbohydrate in the form of dextrimaltose. In addition to the eight groups given margarine, one group was given butter and another, corn oil. Eighty-eight subjects (seventy-seven men and eleven women) completed the dietary period.

In experiment II, ninety-seven subjects (seventy-eight men and nineteen women) ate a fat-free formula diet for eight days. The volunteer subjects were then divided into ten groups as described and continued on rations modified by the substitution of 45 per cent of calories from fat for an equicaloric amount of carbohydrate in the form of dextrimaltose. The brands of margarines, butter and corn oil were the same as those used in the first experiment. Eighty-two subjects (sixty-seven men and fifteen women) completed the dietary period.

RESULTS AND COMMENTS

The results of the first experiment are illustrated in Figure 1 and statistical data are given in Table II.* The increase from 22.5 to 45 in percentage of calories derived from fat brought about by the addition of the margarines in no case led to a significant change, although arithmetic decreases occurred in five of the eight groups. An analysis of variance showed that the margarines did not differ significantly from one another in their effects on plasma cholesterol. The conditions were chosen on the assumption that the diet containing corn oil would exert a significant hypocholesterolemic effect but this did not occur (P, 0.2 to 0.1) and no difference could be demonstrated between the responses to the margarines on the one hand and to corn oil on the other. The only significant change in this series was seen in the group given the additional calories in the form of butter in which a highly significant increase occurred (P, < 0.01). The response elicited by the butter was greater than that for the magarines

* Only one dietary deviation occurred which was considered of significance. This was reported by a subject who ate a chocolate bar and drank a half pint of apple cider on the fifteenth day of the experiment. The results from this individual were similar to those of the rest of the group and consequently they were not discarded.

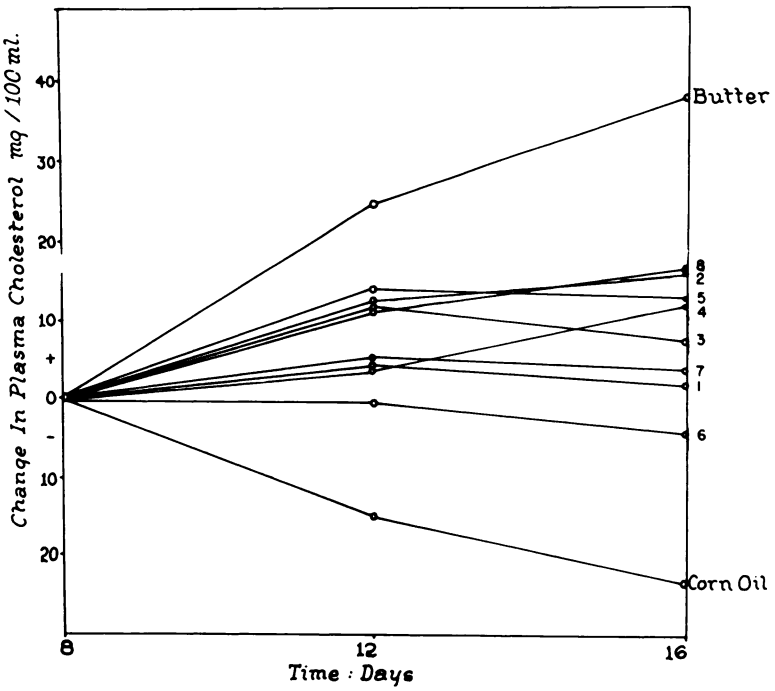

FIG. 2. Average changes in plasma cholesterol values in groups of university students from those observed after eight days on a fat-free diet following substitution of 45 per cent of calories from margarines, corn oil or butter at the expense of an equicaloric amount of carbohydrate (see Table III for numbers of subjects in each group).

(P, < 0.05). Perhaps the most striking feature of these results is the neutral effect that the margarines had on plasma cholesterol levels. Thus the equicaloric substitution for carbohydrate of 22.5 per cent of calories from margarines of widely differing iodine number and fatty acid composition (see Tables IV and V) into a diet already providing 22.5 per cent of

TABLE III

The Effects on Plasma Cholesterol Levels of Substituting Margarines Equicalorically for Carbohydrate in a Fat-Free Diet* at a Level of 45 per cent of Calories

Group No.	Type of Fat	No. of Subjects†	Mean Difference Between Day 8 and Day 16 (mg. cholesterol per 100 ml.)	Standard Error of Difference	t	P
1	Margarine	7	+1.9	5.17	0.36	0.8 –0.7
2	Margarine	7‡	+15.4	2.57	5.97	<0.01
3	Margarine	9	+7.2	5.53	1.31	0.3 –0.2
4	Margarine	8	+12.0	4.45	2.70	0.05–0.02
5	Margarine	9	+12.6	6.32	2.00	0.1 –0.05
6	Margarine	9	−4.5	3.36	1.34	0.3 –0.2
7	Margarine	8	+3.6	5.20	0.70	0.6 –0.5
8	Margarine	7	+16.4	4.13	3.96	<0.01
9	Corn oil	8	−23.1	10.06	2.30	<0.05
10	Butterfat	9	+37.9	3.96	9.58	<0.01

* All subjects had eaten the fat-free basal ration for a period of eight days.

† There were no females in groups 1 and 8, one in groups 9 and 10, two in groups 2 through 7.

‡ One female subject had a very low caloric intake and since her response was radically different from the average of the rest of the group (−23 mg. versus + 15.4 mg. per 100 ml.), the data from this subject were discarded.

TABLE IV
Characteristics of Margarines

Margarine	Iodine Value	Unsaponifiable	Per Cent Trans Fatty Acids
Experiment I (November 1959)			
1	95.6	0.7	20.2
2	84.7	1.0	47.8
3	78.5	0.4	34.0
4	80.4	0.4	35.8
5	70.4	0.3	25.0
6	67.5	0.2	12.0
7	88.5	0.6	39.2
8	85.9	0.4	29.0
Experiment II (March 1960)			
1	95.3	0.6	21.0
2	81.1	0.9	34.8
3	86.6	0.4	30.2
4	80.7	0.5	34.2
5	77.4	0.4	30.8
6	78.4	0.4	32.8
7	91.2	0.5	47.8
8	83.9	0.5	30.8

calories in the form of butter fat did not cause an increase in plasma cholesterol. It should be noted that these conditions, insofar as ratio of animal fat to margarine and total calories from fat is concerned, simulate conditions in practical nutrition.

The results of the second experiment are shown in Figure 2 and statistical data are presented in Table III. Although all the margarines with one exception led to an arithmetic increase compared to the level obtained on the fat-free ration, in only three of these were the P values less than 0.05, margarines 2, 4 and 8. An analysis of variance indicated that there was no significant difference between the margarines. However, the difference between the combined mean of the margarine groups was significantly lower than that of the butter fed group ($P, < 0.01$) and higher than that of the group fed corn oil ($P, < 0.05$). The responses to butter and corn oil demonstrated the usual significant hyper- and hypocholesterolemic effects of these fats.

In a paper published recently, Horlick[12] ob-

TABLE V
Fatty Acid Composition of Margarines*

Margarine	12:0	14:0	16:0	16:1	18:0	18:1	18:2
Experiment I (November 1959)							
1	Trace	0.1	15.4	...	8.4	49.6	26.4
2	...	...	12.7	...	8.4	64.4	12.9
3	...	0.3	16.6	...	7.8	64.3	9.9
4	2.4	1.1	11.8	...	9.1	63.4	11.4
5	3.8	1.6	16.1	...	8.1	62.5	7.4
6	0.1	1.3	23.8	2.0	13.6	50.0	8.5
7	0.2	0.4	13.5	0.3	8.2	60.2	16.9
8	0.1	1.2	15.2	1.4	8.7	57.5	15.8
Experiment II (March 1960)							
1	Trace	Trace	14.8	...	8.1	49.4	27.5
2	...	Trace	13.0	...	6.9	72.5	7.2
3	...	Trace	15.1	...	7.9	63.0	14.0
4	...	Trace	11.6	...	9.3	70.7	8.5
5	Trace	Trace	18.8	Trace	7.5	63.1	10.5
6	...	Trace	15.3	Trace	8.4	66.9	9.5
7	Trace	Trace	14.3	...	6.8	59.8	19.1
8	Trace	Trace	14.6	Trace	7.0	63.5	14.8

* We are deeply indebted to Dr. F. H. Mattson of the Procter & Gamble Company, Cincinnati, for these gas-liquid chromatographic analyses.

tained results of a somewhat similar nature using the same brand of margarine as that designated No. 1 in Tables II and III. The conditions are by no means precisely the same as those used in the work reported here but the data obtained by Horlick indicate that margarines are not as hypercholesterolemic as butter fat. Under the conditions used here the addition of margarines to a fat-free diet at a level of 45 per cent of calories led to a significant increase in three of the eight preparations tested and the addition of margarines at a level of 22.5 per cent of calories to a basal ration providing a similar proportion of calories from butter fat caused no significant change. It may be that the difference in results between Horlick's work and those reported here—and any difference is primarily one of degree—may be due to some uncontrolled factor present in the diet comprised of a variety of foodstuffs consumed by Horlick's subjects. The possible effect of individual variation in the rather small group of subjects participating in the latter's experiments may also be of importance in this regard.

An examination of Tables IV and V showing the characteristics and fatty acid composition of the margarines fails to reveal any clear-cut reason for the differences in response to the ingestion of the different margarines. Furthermore, there was no relationship observed between iodine number and change in plasma cholesterol level.

SUMMARY

None of eight margarines substituted equicalorically for 22.5 per cent of calories as carbohydrate in a basal formula diet providing 22.5 per cent of calories as butter fat caused a significant change in plasma cholesterol in an experiment completed by eighty-eight university students. The responses of the groups which consumed the rations containing the margarines did not differ significantly from that of a group given corn oil but were significantly lower than that of a group given butter fat. These margarines were tested also on another group of eighty-two subjects by substituting them equicalorically for 45 per cent of calories as carbohydrate in a basal formula diet essentially free from fat. Only three groups responded with increases in plasma cholesterol in which the P values were less than 0.05. The difference between the mean of the margarine groups was significantly lower than that of a group fed an equicaloric amount of butter ($P, < 0.01$) and higher than that of a group fed corn oil ($P, < 0.05$). The slight differences in response amongst the margarines could not be definitely attributed to any specific differences in their composition.

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