Saturated fat in the diet and serum cholesterol concentration: a critical examination of the literature

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The concept that the inclusion of so-called saturated fat in the diet is followed by deleteriously high levels of cholesterol in blood serum has been so often stated with positive assurance that it is accepted as fact, not only by the public and practicing physicians, but even by conservative and competent investigators in related fields. Thus, Lees and Wilson (1) in making recommendations for the treatment of type II hyperlipidemia specify reduction of saturated dietary fat as separate from diet cholesterol. The authority quoted by these authors for the recommendation is not a primary source but another review similar to their own (2). It is this practice of referring to secondary or tertiary sources, each taking the last on faith, which has led to the matter-of-fact acceptance of a phenomenon that may not exist.

The message that everyone is in serious danger of coronary heart disease if he does not restrict the amount of saturated fat in his diet is being propagated by every known medium of communication: lectures, newspapers, radio, television, films, and booklets, with the obvious hope that every listener and reader will apply it to himself. If the question were only academic it would not be too serious. Unfortunately, the following on faith of the advice to reduce saturated and animal fat ingestion runs the risk of the consequences of any food fad: extremism and unbalanced diets. Furthermore, in this case it has resulted in adverse economic consequences that could continue and worsen. In addition, it has resulted in the present effort to label foods with their fatty acid composition. If successful, the problems of interpretation by the manufacturers, distributors, and by the consumers will be monumental. If the feared effect of saturated fat is a fact, no sacrifice would be too great to eliminate it from our diet. But is it a fact?

The evidence that saturated fat in the diet is hypercholesteremic even in the absence of cholesterol has been persuasive. The evidence is both epidemiological and experimental, and it involves humans, other primates, various other mammalian species, and classes of animals other than mammals.

It should be noted that almost all the reports upon which the saturated fat theory is based were made in the late 1950's and early 1960's, during the period when diet cholesterol was thought by many investigators to have no effect. That the matter is not a dead issue, however, is evidenced from the constant efforts made to castigate saturated acids, and occasional new papers such as the recent one from the Minnesota group (3). This paper will be analyzed below.

The object of the present communication is to examine the primary sources which have led to the saturated diet fat concept (as distinct from diet cholesterol) in light of our present knowledge, and against criteria of acceptable scientific demonstration. We now

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have the advantage of 10 to 20 years of hindsight and can view far more objectively the data adduced and often too hurriedly published during the days when the subject was new and exciting.

Before embarking on the critical evaluation of the supporting literature, it is necessary to examine the meaning of the term "saturated fat." Chemical terminology restricts the meaning to single bond carbon-to-carbon linkages, a compound being considered saturated if it has no double or triple bonds. Tripalmitin and tristearin are saturated triglycerides and completely hydrogenated natural fats or oils, such as hydrogenated coconut oil, may be called saturated fats. A step away from accuracy and toward confusion is the practice of referring to a natural fat or oil with only a few percentages of polyunsaturated acids as a "saturated fat," ignoring any monoenoic constituents. One might be willing to accept this when the term is used in reference to natural food fats, if limited to such oils as coconut oil or cocoa butter. However, the next step away from accuracy (which is unacceptable) is the now common use of the term as being synonymous with animal fat. It is true that glycerides produced by animals on fat-free diets contain only traces of polyunsaturated fatty acids (4–6). However, animal fats can be quite polyunsaturated if polyunsaturated fats are included in the animals' diets. Eggs, even when high in linoleic acid, maintain their hypercholesteremic property (7) because of their constituent cholesterol.

The misleading practice of referring to animal fats as "saturated fats" has arisen in epidemiological studies because only natural foods such as butter, cheese, and eggs are involved in population investigations. Although such studies from a few laboratories have had disproportionate influence in support of the theory, there are probably more epidemiological reports to the contrary. In spite of the influence of epidemiological studies on the saturated fat theory, they will not be reviewed here whether they support the theory or refute it. It is incorrect to quote epidemiological data as tests of the hypothesis that saturated fats per se are hypercholesteremic. Epidemiological studies can show simple relationships but cannot prove cause and effect relationships because of many uncontrollable and often unsuspected factors, any one or combination of which may be the causative agent. On the other hand, the relationships they do expose are important if viewed in proper perspective, and can be the point of departure for controlled experimentation.

In addition to the confusion and misunderstandings brought about by misuse of terminology, there are a large number of variables, lack of control which make interpretation difficult if not impossible, and certainly inconclusive. These will be brought out with each paper reviewed, it being adequate here to note some of the more common: 1) failure to consider the effects of plant sterols and cholesterol, 2) attribution of differences between saturated and polyunsaturated to the former when the effect could be due to the latter, 3) inadequate time allowed to reach a steady state before change in diets, 4) lack of attention to food intake and weight gains, 5) lack of control over "free" subjects' adherence to the test diets, 6) the effect of hydrogenation on the phytosterols in hydrogenated oils that could destroy their effect on cholesterol absorption, and 7) inadequate numbers of subjects.

Bloor (8) observed some of these errors in a review of the literature from 1914 to 1939, and concluded that, "From the variable nature of the results reported, the occurrence of a characteristic increase in blood cholesterol after fat feeding is doubtful." Bloor observed an oversight in the early work that has often been repeated since. "Few of these workers paid any attention to the cholesterol content of the fat fed..." As will be examined in detail below, during the 1950's this was no oversight, the consensus during that period being that diet cholesterol plays no role in serum cholesterol concentration; moreover, those studies of the 1950's are the foundation of the saturated fat concept.

In a review of earlier work, Rosenthal (9) wrote: "As mentioned by Hoppe-Seyler in 1857 and verified by Versé, Schoenheimer, Tannhauser, Burger and others, neutral fat paves the way for cholesterol absorption," thus leading to the explanation of why "diets high in cholesterol and low in
neutral fat may result in much lower blood cholesterol than a diet high in neutral fat and low in cholesterol.” Thus, the early workers learned what more recent investigators seem to have forgotten, but a fact to which we must return: the role of neutral fat is indirect and secondary to that of cholesterol, probably only to the degree to which it contains cholesterol and affects cholesterol absorption.

Because of the great variations in the details of lipid metabolism between classes and species of animals, this critique is concerned mainly with humans. References to studies with other animals are limited to supplementary observations.

Due to the great volume of literature, all studies on the subject cannot be evaluated here. It is hoped that those most influential in establishing the saturated fat hypothesis are included, excluding epidemiological studies and most studies with free-living subjects on practical diets, such as the Framingham Study, as these are not tests of the hypercholesteremic properties of saturation. However, some epidemiological and mass studies, which used cholesterol-containing fats and purported to demonstrate that saturated fats are hypercholesteremic, will be examined in order to demonstrate how such diets cannot be used to test the role of saturated fat per se.

Hydrogenated vegetable oils

Hydrogenated vegetable oils have been accused by association of causing hypercholesteremia. Once the saturated fat theory became fixed in people’s minds, any saturated fat was assumed to be guilty. However, the experimental data testing the hypercholesteremic powers of hydrogenated oils are weak and susceptible to alternative interpretations.

An uncontrolled possibility, which could explain reported differences in serum cholesterol concentration due to the ingestion of hydrogenated versus the natural vegetable oils, is the effect of the hydrogenation on phytosterols. That phytosterols reduce serum cholesterol concentration has been adequately documented and the literature reviewed (10). Yet the possibility that hydrogenation may destroy this effect appears not to have been considered. Even the demonstrated effect of the natural phytosterols is more often ignored than acknowledged. In the following analyses of individual reports these alternative interpretations will frequently be pointed out.

It is not enough to answer that in practical situations it may make no difference where the fault lies as long as the substitution of natural vegetable oils for saturated fats (from whatever source) will have beneficial effects. However, if the responses are due to the sterols, the castigation of saturated fats, per se, results in distortions such as the attempted labeling regulations for fatty acid composition, and even in the development of food phobias by impressionable people. If saturated acids are neutral, as in cocoa butter, they should be no more singled out than oleic acid.

A possible hypocholesteremic effect of polyunsaturated fatty acid-containing oils is not ruled out and may be a valid concept. The reviewer himself has data demonstrating more rapid turnover of cholesterol in tissues of rats on high linoleic acid versus saturated fat diets. Polyunsaturated fatty acids are preferentially catabolized (11) and are incorporated into the number 2 carbon of glycerides, especially lecithin, and thus become esterified to cholesterol. Also, dietary linoleic acid has a far more inhibitory effect on lipogenesis than does saturated fat when ingested with sugar (12, 13).

It is reasonable to assume that unsaturated phytosterols, such as the ubiquitous β-sitosterol, are hydrogenated along with the fatty acids and lose their hypocholesteremic property. Thus, hydrogenated oils could facilitate enterohepatic circulation of cholesterol, whereas the normal sterols in natural oils could inhibit it.

Scrutiny of the studies in which serum cholesterol response to hydrogenated vegetable oils has been recorded impresses one with the slight differences observed, almost all of which could be just as readily attributed to normal variation in the subjects, a factor that is usually inadequately determined.

A) In what was probably the first test of hydrogenated oils, Bronte-Stewart et al. (14) fed one Bantu subject 100 g of hydrogenated peanut oil for 12 days after 10 days on a corn meal, white bread, and casein reference
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diet. Although there was only one subject in this study, it will be examined in some detail for two reasons: It set the stage for the case against hydrogenated oils, and it contains some typical errors. The most critical error lies in the interpretation of line graphs in which the changes during one period are not related to the overall trends. As in this case, the maximum or minimum values during a period are often pointed out as representative. In some cases, the average values are used and in still others the final values of the periods are considered the representative ones.

From the 1st to the 7th day on the hydrogenated peanut oil test diet, the serum cholesterol of this subject rose from 130 mg/dl to 160 mg/dl, the latter value being taken as representative of the response. But by the 12th day on the same test diet the serum cholesterol had fallen back to 130 mg/dl, a point the authors overlooked. It continued to fall in almost a straight line to 120 mg/dl during the subsequent natural peanut oil diet period. Thus, the changes cannot be attributed to differences in responses to the two oils, but more likely, were normal fluctuations following the removal of the subject's normal cholesterol-containing foods. During a 2nd period on the hydrogenated oil, serum cholesterol reached a maximum of 145 mg/dl. Without controls, the best interpretation of these data is that the serum cholesterol values represent normal variations, or that the differences represent temporary responses to diet changes attributable to almost anything, from changes in other constituents of the diets to changes in intestinal status. They could also be explained by the hydrogenation of phytosterols or any combination of these factors.

The same subject was given 15 g/day (8% of total calories) of the saturated fraction of sunflower oil after 14 days on the basal diet. No analytical datum on the oil is given, although it is referred to as a "palmitate-stearate mixture." The serum cholesterol concentration sharply rose to 180 mg/dl. The pronounced response to so little "palmitate-stearate" fat is mysterious. If this fraction was a "palmitate-stearate mixture," it would have been poorly absorbed. One suspects that it probably contained a fairly high level of oleate that would make it similar to cocoa butter, which admittedly does not cause an increase in serum cholesterol concentration. Furthermore, there was already approximately 3% polyunsaturated fat in the basal diet from corn and wheat. One suspects unknown factors, such as the surreptitious consumption of high cholesterol food by this uneducated Bantu who was not isolated but continued with his normal daily routine.

B) A classical, and perhaps the most quoted report of the effect of the degree of saturation of diet fats on serum cholesterol concentration is that of Ahrens et al. (15). Published in 1957 during the high point of development of the saturated fat theory, and utilizing only three subjects to test the effects of hydrogenation, it is still widely quoted in defense of the theory in spite of the fact that the authors stated "...none of the experiments performed here or elsewhere has ruled out the possibility that the factors sought may lie in the nonglyceride portion of the fed fats." The senior author has reiterated that, "...in my opinion there is no data including my own" (mentioning this study) "which establish with certainty that the phenomenon" (control of serum cholesterol by dietary fat) "is due to fatty acid differences" (16).

This paper is reviewed in detail here because it is so often quoted and because typical oversights are again included.

In one study, three liquid formula diets (17) containing normal corn oil or one of two of its hydrogenation products were fed as 40% of the calories. Before the test diets, the subjects were standardized on a 40% (calories) corn oil formula diet. Each diet was continued until a "steady state" was reached, defined as that period during which "the standard deviation of the mean level of total cholesterol, as well as phospholipid, was less than ± 20 mg per 100 ml serum." It was found to take 2 to 3 weeks to reach this steady state.

Three patients were used. Patient 20 had hyperlipemia with atherosclerotic heart disease and healed myocardial infarction but was "free from conditions which might complicate...metabolic studies." Serum cho-
Lesterol assays were made weekly. The two hydrogenated corn oils of iodine values (IV) of 58 and 80 and the natural corn oil, IV 126, were tested in that order.

The initial serum cholesterol concentration of patient 20 on an ad libitum diet, as estimated from a line figure, was approximately 350 mg/dl. This dropped to a baseline value of 260 mg/dl on the control corn oil diet. A peak of nearly 310 mg/dl (estimated from the figure) was reached after 5 weeks on the IV 58 hydrogenated oil diet. However, the concentration fell back to approximately 295 mg/dl at the 7th week and continued downward on a straight line during the rest of the time on that diet to a minimum value of 275 mg after 3 weeks on the IV 80 oil diet. Furthermore, there was a short-lived increase during the 6th and 7th weeks of this new diet to nearly 290 mg/dl.

Thus, the final serum cholesterol values at the end of the two diet periods were 295 mg/dl and 290 mg/dl. One must conclude that there was no difference in response to the two oil diets. Obviously, the greatest differences were normal variations not related to diet oil.

The authors, however, in a table, report the respective values as 298 and 273 mg/dl and consider the differences significant. Obviously, they used averages, but as the values were steadily changing (though within the limits of $\pm 20$ mg/dl), one must consider that the final concentrations, not the averages, more truly represent the physiological responses.

The formula was then changed to one containing unhydrogenated corn oil of IV 126. The serum cholesterol concentration continued its overall decline to about 210 mg/dl in 2 weeks, no value being given for the 1st week. It fluctuated between that value and 190 or 195 mg/dl through the 6th and 7th weeks on this diet.

Perusal of the line graph raises the question whether this patient might not have followed the same course by substitution of any cholesterol-free diet for the ad libitum one because there is a reasonably steady drop in the values from the middle of the highest hydrogenated oil period to the end of the period on natural oil, the one temporary increase occurring during the ingestion of the intermediately unsaturated oil following the more highly hydrogenated one. Note, also, that the base-line value of 260 mg/dl and the final value of 190 mg/dl are both "steady-state" responses to natural corn oil. One must conclude, therefore, that no effect can be attributed to the hydrogenated oils.

Another difficulty with this study is that the authors, in discussing the response of this patient say, "...the feeding of corn oils hydrogenated to iodine values of 80 and 58...produced progressively higher levels of cholesterol...in the serum." This is worded because the oils were fed in the reverse order and were followed by progressively lower levels of serum cholesterol. The distinction is critical, for if the oils had been given in progressively higher degrees of saturation and the serum cholesterol had risen as stated, the effect of removal of the ad libitum diet cholesterol would have been controlled. In the order they gave, it was not.

Using any criterion of evaluation of data to demonstrate a phenomenon, these data give no support to the theory of a hypercholesteremic property of hydrogenated vegetable oils. Yet the authors state that "the most clear cut response" (of all their data) "was shown by patient 20."

Patient 30, with cardiac neurosis (sic), was given the corn oils of IV 126 first and then the IV 80 after the control period on the corn oil formula. The corresponding serum cholesterol values, given in a table, are 119 and 125 mg/dl, which are approximately equal, supporting the reviewer's interpretation that the changes found with patient 20 were due to slow recovery of a hypercholesteremic person from the high cholesterol-containing ad libitum diet, and not to saturated fatty acids.

The third patient, 18 (with "hypercholesterolemia," "arteriosclerotic heart disease," and "myocardial infarction"), was given the corn oil diet followed in order by natural and hydrogenated cottonseed oils of IV 106 and 68, respectively. The resultant serum cholesterol values were $186 \pm 12.9$, $177 \pm 11.2$, and $217 \pm 7.6$, respectively. Note that the level reached after the natural cottonseed oil is the same or lower, than after corn oil.
All these values are low and probably the normal variation for this subject. It is impossible to know without proper controls.

It is thus clear that, on close examination, the data in this paper from only three subjects, none of whom are normal, cannot be justly referred to as a demonstration of a hypercholesteremic response to hydrogenated vegetable oils, a conclusion the authors themselves fail to claim.

C) A study often quoted to buttress the theory that hydrogenated oils are hypercholesteremic is that of Malmros and Wigand (18) who compared nonhydrogenated and hydrogenated whale oil with hydrogenated coconut oil and natural corn oil.

Nine “healthy” volunteer subjects with average serum cholesterol concentrations of approximately 220 to 230 mg/dl on an ad libitum diet (estimated from a line chart in which the scale is 2 mm for 100 mg/dl) continued at that level or rose to nearly 240 mg/dl when given 40% of their calories as hydrogenated coconut oil added to a low fat diet of vegetable products. The substitution of whale oil for the coconut oil resulted in a steady drop to approximately 200 mg/dl after nearly 10 days. Without controls or standard deviations, the relative significance of the 20 mg/dl difference between the ad libitum diet and the whale oil diet is questionable. The substitution of corn oil provoked further decreases to almost 180 mg/dl during a 1-week period, analyses being made after 3 (?) days and the end of the week.

A corresponding study was made with 12 “healthy” volunteers but hydrogenated whale oil was used instead of unhydrogenated. In this case, the average serum cholesterol value on ad libitum food selection was just below 220 mg/dl, rising to approximately 230 mg/dl during 1 week on the hydrogenated coconut oil diet and subsequently remaining there during 2 weeks on the hydrogenated whale oil diet. There was thus only normal variation in serum cholesterol during these three periods.

Substitution of corn oil again resulted in a rapid drop to 200 mg/dl in 1 week.

The difference between the response to hydrogenated (230 mg/dl) and natural whale oil (200 mg/dl) is suggestive. But it is questionable whether there is any difference between the ad libitum and either of the whale oil diets in the individual trials. It is thus incorrect to compare the whale oils in different trials.

A probable explanation for the different responses to the two oils is that 40% of the calories of the diet with the whale oil containing approximately 200 mg cholesterol/100 g (ca. 900 kcal) would amount to the ingestion of 667 mg cholesterol in 3,000 kcal of daily food intake. Whale oil contains as much cholesterol as beef tallow, i.e., nearly 0.18% ((19), and unpublished analyses by the reviewer). This is equivalent to 2.5 egg yolks, considered by many as being the maximum that can be absorbed. This easily explains why hydrogenated whale oil did not change serum cholesterol relative to ad libitum food selection. The authors explain that the natural whale oil was rancid, and that 3 of 12 subjects could not stand the smell and dropped out of the experiment because of this and diarrhea. Although it is said that the remaining nine “consumed the entire fat ration,” one is inclined to wonder. No datum is given on food consumption of these unrestricted volunteers. If the sickening stench of rancid whale oil did restrict their food intake, as should be expected, and did result in reduced fat and cholesterol absorption as well as an increase in fecal excretion, the observed drop in serum cholesterol would be explained.

The authors did not claim that their data proved a hypercholesteremic quality of the hydrogenated products, but suggested that “the cholesterol depressing effect of certain fats is related to their content of polyunsaturated fatty acids. Investigation of this possibility requires further experiments with pure esters of the different fatty acids.” Obviously, the data cannot be used as demonstration of a hypercholesteremic propensity of hydrogenated whale oil.

Considering the lack of knowledge of normal variation in these subjects, disregard of the effects of cholesterol and of sitosterol and its hydrogenation product, and the dearth of reliable food intake data, one must conclude that this study is not a demonstration of a
hypercholesteremic property of hydrogenated or saturated fat.

D) Beveridge and others, in two reports (20, 21), have contrasted the response of students to natural and partially hydrogenated corn oil after adjustment to a 35% butter oil diet and to a fat-free diet.

Although the authors did not claim that these studies demonstrated that saturated fat is hypercholesteremic, they contain several typical oversights representative of reports on the subject and are, therefore, examined in detail in order to demonstrate these oversights and point out why credence cannot be given to conclusions from such reports.

In the first study (20), the objective was to assess the importance of sitosterol in the reduction of serum cholesterol effected by corn oil when added to a fat-free diet, in contrast to the increase that occurred upon the addition of butter to the same fat-free diet.

In one study (20), 57 male and 5 female students were placed for 8 days on a homogeneous formula diet containing 35% of its calories as butterfat. They were then divided into subgroups, one of which received 25% of calories as corn oil for 8 days at the expense of carbohydrate. Another group consisting only of males, received hydrogenated corn oil containing 21.7% saturated acids, 73.5% "oleic acid," and 0.38% dienoic acids. The data show that 46.2% of the double bonds were trans and 53.8% cis. Thus, approximately one-half the monoenoic acid was elaidic and not oleic. The males who ingested corn oil had an average of 18.3% less serum cholesterol at the end of the 8 days than at the beginning. The corresponding value for the females was -23.4%. After the hydrogenated corn oil diet, the response of the males was -22.7%. Thus, there was no difference in response to the two oils.

In a second study, the same authors (21) gave the natural and hydrogenated corn oils as 45% of the calories for 8 days to two groups of students who had been on a fat-free formula diet a previous 8 days. A control group was continued on the fat-free diet for the second 8-day period, after which their serum cholesterol had increased an average of 2.4% from the end of the 1st period. The serum cholesterol concentration of those who consumed the corn oil decreased 16.4%, whereas it increased 2.4% in those who consumed hydrogenated corn oil.

Note that in the second study, the saturated and elaidic acids and the hydrogenated phytosterols of the hydrogenated oil had no effect at all, the serum cholesterol remaining at the same level as that of the group which continued on the fat-free diet. The response to the natural oil could be explained by the normal serum cholesterol-lowering effect of its constituent sitosterol and possibly to the unsaturated acids. This is one of the rare studies in which the saturated and polyun-saturated fats are compared with a neutral diet rather than to each other, so that each can be assessed independently.

It is unfortunate that a sitosterol-free oil, such as sesame or a stripped oil, was not used as a control. There are also other weaknesses that make definitive conclusions impossible:

1) There is no record of food intake. Certainly on the 60% fat calorie diet there was less formula consumed than on the 45% fat calorie diet.

2) The hydrogenated oil contained 21.7% saturated acids (individual acids not given), 73.5% oleic acid, and only 0.38% diene fatty acids. The data showed that 46.2% of the double bonds were trans. As the double bonds were almost all in the monoenoic group, over one-half of the oleic was probably elaidic. The "oil" had a melting point of 41 to 42 C. When added to a fat-free diet, this oil and its constituent phytosterols were certainly poorly absorbed. But when mixed with butter in a 25:35 ratio of oil to butter, it was probably better absorbed and its phytosterols with it. The oil might also have aided in the reabsorption of endogenous cholesterol. This by itself can explain why the response to this highly hydrogenated oil was no different than to the fat-free diet to which it was added (at the expense of carbohydrate). However, there was a response equal to that of natural oil when it was given with more than its own weight of butter.

3) It is also questionable whether one can justify calculating percentage differences between means of a group of 5 to 10 free-living students, who have widely different serum
cholesterol concentrations on an ad libitum diet, after only 8 days on a test diet.

Here again is an example that contrasting conditions alone only demonstrate differences but do not place responsibility. Hydrogenation may remove hypocholesteremic substances in an oil, but that does not mean that it adds hypercholesteremic substances.

Thus, the best one can conclude from these studies is that there is no difference in response to natural and hydrogenated corn oil, except as can be accounted for by the secondary effects of the nature of diet triglycerides on sterol absorption.

E) A study which gave weight to the saturated fat theory, but which demonstrates a number of the pitfalls of investigations on this subject is that of Gordon and Brock (22). Hydrogenated sunflower seed oil was added to the control diet of one person. Evidently, the control diet was the man's normal diet during which his serum cholesterol concentration varied from 179 to 229 mg/dl. The fat was "melted and drunk or combined with food." Supposedly, 75 g of the fat was ingested daily from the middle of March to late in June as estimated from a line figure, during which time his weight increased from approximately 162 to 168 lb. His serum cholesterol concentration increased from 210 mg/dl to nearly 240 mg/dl during that period. There was a discontinuation of the supplement until late in July, during which time his weight underwent a slight decrease, the serum cholesterol value returning to less than 220 mg/dl.

Whether one can consider the rise and fall in serum cholesterol during the ingestion of hydrogenated sunflower seed oil to have been a physiological response to the saturated fatty acid in the oil is moot, considering that the figure relates to only one subject, that the fat was taken "neat" once a day, and that in this subject the serum cholesterol concentration during the control period varied within a 50-mg range which included all test values.

F) Horlick (23) gave student volunteers a low (4%) fat diet for 1 week after a control period on a 45% fat, 315-mg/day cholesterol diet. The serum cholesterol concentration dropped an average of 20%. Upon the addition of corn oil as 40% of the calories, the serum cholesterol remained unchanged. However, upon the addition of hydrogenated fat as a special corn oil margarine or a commercial margarine instead of natural corn oil (each as 40% of the calories), the serum cholesterol value returned in 3 weeks to the original control diet level, or only almost halfway to the control level in some experiments.

Here again, all the effects can be attributed to phytosterols in the natural oil and their saturation in the hydrogenated oils. Such experiments do not prove a hypercholesteremic effect of saturated acids. No positive hypercholesteremic faculty of hydrogenated acids was demonstrated even if one conceives that hydrogenation did remove possible hypocholesteremic polyunsaturated fatty acids.

It should be borne in mind that fat-free diets are questionable base-line or reference diets for comparing the responses with two or more oils. In such dietary conditions lipogenesis is stimulated, lipid metabolism is increased, serum triglycerides are increased along with changes in the serum lipoprotein picture, and fatty livers may even develop, possibly affecting hepatic sterol metabolism and enterohepatic circulation.

G) Anderson et al. (24) fed matched groups of schizophrenic men a normal American diet, which they called the "house diet," followed by successive periods of 21 days on various fats and oils in a design calculated to "compensate for any general time trends."

In experiment "K," 30 g of safflower oil or hydrogenated safflower oil were substituted for 68 g of carbohydrate in the house diet. The total diet fat was 46 g/day; therefore, 16 g was from the normal house diet. The amount of cholesterol in the house diet fat is not given, but it must have been appreciable because the authors say that the diet provided "about 40% of the total calories from fats and being generous in meat and dairy products." Animal fat contains approximately 0.2% cholesterol and almost 1.5% butterfat. In one group of 14 men, the hydrogenated safflower oil diet was followed by the natural safflower oil diet. In another group of 13, the oils were given in the reverse order. Combined data give the mean serum cholesterol values as: safflower oil diet 196 ± 6.4 mg/dl, hydrogenated safflower oil diet 206 ± 6.0 mg/dl.
mg/dl, the difference being 10 ± 2.4. Considering the normal variations for which datum is unavailable, these values are not significantly different. The natural safflower oil contained 12% saturated acids, 10% monone, and 75% polyene. The corresponding values for the hydrogenated oil were 32, 55, and 13%.

In another experiment “N,” the serum cholesterol after 21 days of natural safflower oil ingestion was 160 ± 6.2 mg/dl (standard error) and 185 ± 6.2 mg/dl after the hydrogenated oil. The data in experiment K give evidence that saturated acids from hydrogenated oils are not hypercholesteremic, as well as evidence against a hypocholesteremic property of polyunsaturated oils. From the data in experiment N, 185 mg/dl cannot be called hypercholesteremia, even if the differences between the two values were significant. But even that cannot be judged as there was no control for normal variation. Such slight differences at these low levels have doubtful physiological significance.

Onus is also removed from the hydrogenated fatty acids by realization that the small responses that were obtained may have been due to the constituent cholesterol, which in experiment N was derived from 12 g of butterfat and 23 g of beef and pork fat, probably approximately 100 mg in all.

The data in this study can more readily be used as evidence against the saturated fat theory than for it.

H) In one study (25), groups of 4 male and 4 female college students were given test meals containing 35% fat calories, 78% of which was from either cottonseed oil or hydrogenated cottonseed oil. The former contained 50% linoleic acid, the latter 4.3%. The saturated fatty acid content was not given, though the respective iodine values were 107 and 60. After 15 days on the test diets (following ad libitum food selection), the average serum cholesterol levels were 160 and 164 mg/dl, respectively.

These data, of course, weigh against the saturated fat hypothesis.

I) Two experiments were conducted with sunflower seed oil and hydrogenated sunflower seed oil in a study with Bantu and white prisoners (26). The respective linoleic acid compositions were 64 and 1.6%, stearic acid 4 and 17%, and palmitic acid 7.5 and 7.8%. The rest was oleic (sic). No values are given for trans or position isomers.

In two experiments, the natural and hydrogenated oils were given to the prisoners for 22 and 29 weeks, respectively, as 25% of a basal vegetable diet and containing 15% of the calories as fat. Thus, the test diets had approximately 40% fat calories, all from vegetable sources.

The average serum cholesterol levels at the end of the test periods on the natural and hydrogenated oil diets were 160 ± 25 mg/dl and 210 ± 40 mg/dl, respectively, in the whites and 147 ± 16 mg/dl and 199 ± 24 mg/dl in the Bantu.

Even if the higher values do not represent hypercholesteremia, they are impressive evidence that hydrogenated sunflower seed oil produces higher serum cholesterol levels than the natural oil. However, it is not stated how well each diet was accepted, whether there were weight changes or, most importantly, whether the prisoners were able surreptitiously to obtain the high cholesterol food they were more accustomed to eating.

Had a few more of the 39 prisoners on the hydrogenated oil than the 38 on the natural found access to the customary high cholesterol food of the Bantu, the suspicion of “significance” of the data could be explained away.

Also, hydrogenation of the plant sterols or loss of linoleic acid can explain the loss of hypocholesteremic activity by the hydrogenated oils, but hypercholesteremic activity cannot be laid at the door of the saturated acids. Besides, there was no hypercholesteremia.

J) In a study in which the authors interpreted as evidence against the saturated fat theory, Beveridge and Connell (27) fed 78 men and 19 women students a basal diet mixture of skimmed milk, sucrose, dextromaltose, and a vitamin mixture for 8 days. The subjects were then divided into 10 groups of 8 to 10 each and were adjusted as to their serum cholesterol levels on the 4th day. Eight of the groups each received a different margarine as 45% of the calories, substituted for dextromaltose. The iodine value of the
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margarines varied from 67 to 96, and the constituent trans acids from 12 to 48%. The saturated acids varied from 21 to 34% and the linoleic (18:2) from 7 to 26%. The ninth group received 45% of the calories as corn oil, and a 10th group received 45% of the calories as butter.

The serum cholesterol responses to the added fats and oils were compared with the level after 8 days on the basal diet. Eight days of the corn oil diet resulted in serum cholesterol concentrations 23% below those on the low fat basal diet, and the butter diet almost 38% above.

The margarines gave values from −4.5 to +16%, none statistically different from the response to the basal ration, leading the authors to conclude that the responses to the corn oil and butter were due to their sterol content and not to any difference in their fatty acid content. The total unsaponifiable matter of the margarines varied from 0.4 to 0.9%, the source of the products being unknown. Corn oil is composed of nearly 2% sterols. In addition, as explained elsewhere, hydrogenation may destroy the action of the vegetable oil sterols.

This paper and other similar reports from this laboratory (20, 28) present strong evidence against the theory that hydrogenated vegetable oils or saturated fats are hypercholesteremic.

K) In an ambitious and carefully carried out study with prisoners, isolated so that one may have confidence in the recorded dietary regimens, McOsker et al. (29) compared the effects of butter, 4 partially hydrogenated vegetable oils, winterized cottonseed oil, and a blend of animal and vegetable fats designed to mimic the fat mixtures consumed “in the average American diet.” The fats were homogenized as 20.6% of the dry weight into a mixture of dried egg white, dextrose, salts, and vitamins. Coffee, fruit, and vegetables supplemented the formula diet.

There were 7 groups of 7 persons each, and four periods of 8 weeks each. Each group received four different fats, one during each period. Thus, all groups did not consume all fats. The hydrogenated fats and cottonseed oil contained from 14 to 26% saturated acids and from 13 to 56% polyunsaturated acids. All fats contained the same total amount of sterols. The amount of cholesterol in the reference fat was not given.

Because all groups were not tested with all fats, each fat was evaluated according to the average serum cholesterol it effected over the entire experiment. It was said, “that in order for the difference between any two adjusted mean serum cholesterol levels to be significant at the 95% confidence level, the difference must be at least 12 mg/100 ml of serum.” By this criterion the cottonseed oil and the four partially hydrogenated oils were equal, the serum cholesterol responses being between 158 and 167 mg/dl. The mixed animal and vegetable fats produced a serum cholesterol average of 175 mg/dl and the butter 204 mg/dl.

The serum cholesterol concentrations in this study are thus directly related to the cholesterol levels in the oils or inversely related to the plant sterol levels.

Thus, hydrogenation did not produce saturated acids with hypercholesteremic activity, nor do the polyunsaturated fatty acids have hypocholesteremic attributes.

L) DeLongh et al. (30) designed a study to test for possible hypercholesteremic action of isomers of unsaturated fatty acids. Actually, as performed, the study contrasted a series of mixtures with respect to their effects on the serum lipids.

The subjects were 72 healthy male oligophrenics. The test fats were given as 33% of the calories, included partially in pastries and “special milk” in a diet of lean meat. The other constituents were not mentioned, except that cheese, eggs, and fish fat were excluded.

Test periods were 4 to 6 weeks. The fats tested were various mixtures of sunflower seed oil, hydrogenated whale oil, hydrogenated soybean oil, natural soybean oil, hydrogenated fish oil, margarine, butterfat, “monoglycerides,” and “triglycerides.” The fatty acid composition of the oils and mixtures are given.

The experimental design is not given completely. Perhaps all patients received the fats at the same time in the same order. The four tests with sunflower seed oil, following 1) a normal diet, 2) the two hydrogenated soy-
bean oil diets, and 3) the butterfat diet, were accompanied by serum cholesterol levels of 157, 149, 161, and 168 mg/dl, respectively. Eight other fats and mixtures gave serum cholesterol levels between 164 and 175 mg/dl. One test with hydrogenated whale oil resulted in a serum cholesterol concentration of 186 mg/dl and one of hydrogenated soybean oil of 196 mg/dl.

A calculation was made to test the relation of fatty acid composition with the serum cholesterol prediction obtained by Keys' equation (31). It was concluded that the elevating effects were much smaller than the prediction. As a matter of fact there was no relationship whatsoever, as the variations on the sunflower seed oil diet varied from 149 to 168 mg/dl, the last value apparently being incomplete recovery from the preceding butter diet that produced a level of 198 mg/dl, due of course, to its cholesterol.

In addition to the comment concerning lack of correlation between the saturated fatty acid composition of the oils and the serum cholesterol levels as predicted by Keys, the authors found that the trans acid effect was "very small." Actually, there was no significant serum cholesterol elevating effect whatsoever due to saturated acids though the authors conclude that, "The results suggest a relatively strong serum cholesterol lowering effect of the first 10% linoleic acid in the dietary fat when linoleic acid replaces monoenoic acid."

These papers on the response of serum cholesterol to hydrogenated fat were selected as being the most representative and influential in establishing acceptance of the saturated fat theory. Yet of the 12 papers reviewed, the data from at least four (25, 27, 29, 30) are actually contrary to the saturated fat theory. The data of the rest are suspect because 1) they ignore the effect of constituent sterols; 2) they lack controls, especially of normal variation of serum cholesterol in the individuals; 3) often they do not allow adequate time to establish a steady state; 4) there is questionable control of the subjects' adherence to the diets or of the amount of food intake and weight gains; or 5) they do not use adequate numbers of subjects.

Another factor which cannot be ignored is that in these studies, the highest serum cholesterol values attributed to saturated fatty acids are all low, with none in the hypercholesteremic levels.

Also, even in studies in which hydrogenated fats are compared with unsaturated fats, differences cannot be attributed to the effect of the saturated acids unless possible effects of the polyunsaturated fats are controlled. The differences could all be due to the polyunsaturates (plus their phytosterols) and not to the saturated fats. Although Beveridge pointed this out in 1958 (21), it has usually been overlooked, even by Beveridge himself (20).

**Cocoa butter**

Almost all studies using cocoa butter have found it neutral with respect to its effect on serum cholesterol concentration. Cocoa butter is a much more fair test of the saturated fat theory than coconut oil because its fatty acids are the same as those in animal fats and hydrogenated vegetable oils. It contains approximately 24% palmitic, 35% stearic, 38% oleic, and 2% linoleic acids. Coconut oil is a highly unusual mixture containing approximately 45% lauric acid, which may be toxic in such high concentrations. (See below in the coconut oil discussion.)

Most of the studies with cocoa butter are more recent and of better design than those with coconut oil, which may account for the difference in findings relative to the two fats. Such explanation has more validity than the one offered for cocoa butter by Keys et al. (32) that "Stearic acid, as well as saturated acids containing fewer than 12 carbon atoms have little or no effect on serum cholesterol in man." The palmitic acid of cocoa butter cannot be thus ignored. It is pertinent that the relative response to saturated 12, 14, and 16 carbon acids is also controversial, even among the supporters of the saturated fat theory (33, 34).

Ahrens et al. (15), in their oft-quoted study compared responses to cocoa butter with those to butter and corn oil by a 52-year-old "arteriosclerotic heart disease" patient. On an ad libitum diet, the serum cholesterol concentration of this subject was 194 mg/dl. Butter at 40% of the calories
SATURATED FAT AND SERUM CHOLESTEROL CONCENTRATION

for 6 weeks caused the serum cholesterol to increase to 219 mg/dl. Immediately after replacement of butter with cocoa butter, it promptly fell to 200 mg/dl, at which level it remained for 5 weeks, increasing to 259 mg/dl upon the return to butter, and quickly dropping to 211 mg/dl upon substitution of cocoa butter a second time. Corn oil was then given, and the serum cholesterol started to drop at once, reaching approximately 175 mg/dl in 4 weeks, apparently still falling at the end of the study.

One can agree that the ingestion of cocoa butter produced serum cholesterol concentrations somewhere between those effected by butter and corn oil. But these intermediate values might well have been reached by the ingestion of a neutral reference oil on the assumption that corn oil is hypocholesteremic due to its sterols and that the cholesterol in butter is hypercholesteremic. Thus, this study actually shows that cocoa butter is neutral in its effect and not hypercholesteremic. Also, 200 mg/dl is not a hypercholesteremic value.

B) In an early study using a large number of hydrogenated and natural vegetable and animal fats, including whole oil and cocoa butter, Malmsros (35) compared cocoa butter with an ad libitum diet.

The cocoa butter was given as 40% of the calories added to a basal diet of potatoes, rice, bread, cereals, vegetables, fruit, and sugar. During a 3-week period after the start of the test diet, the serum cholesterol concentrations slowly decreased from approximately 225 mg/dl on the free diet to almost 150 mg/dl, a convincing demonstration that cocoa butter is not hypercholesteremic.

C) In a study (36) in which cocoa butter was contrasted with corn oil, the subjects received a "normal" diet of lean meat, fish, and potatoes, rather than a formula diet. Pastry goods and sweet biscuits were prepared with the fat under examination. The diets contained 40% fat, 10% protein, and 50% carbohydrate.

In a pre-experimental (normal) diet, serum cholesterol levels varied among the subjects from 132 to 344 mg/dl ± 16 mg/dl.

After 10 days on the corn oil diet, the average serum cholesterol concentration dropped from 215 mg/dl to 163 mg/dl at approximately which level it remained for nearly 7 months. The amount of reduction in each individual averaged 26% of the initial value, the absolute drop thus being directly proportional to the original level.

Substitution of cocoa butter alone or with corn oil caused the serum cholesterol to increase, the increase being proportional to the decrease in the amount of corn oil, or alternatively, to the increase in cocoa butter. Return to corn oil alone again resulted in prompt reduction in serum cholesterol concentration.

It is indeed unfortunate that in this study, there was no neutral oil group or control period as the serum cholesterol concentrations are obviously resultants of the relative effects of the two diet oils. One cannot know how much weight to ascribe to each, but all the changes may be attributed to the constituents of the corn oil, in which case one can conclude that cocoa butter is neutral.

D) Erickson et al. (37) reported studies in which seven groups of six male prisoners were each given 41% of their calories as various fats and fat mixtures in formula diets. The fats were: 1) partially hydrogenated soybean oil; 2) a mixture of olive oil, safflower oil, and cocoa butter but resembling #1 in fatty acid composition; 3) a mixture of #1 with cocoa butter; 4) a mixture of #2 with cocoa butter; and 5) cocoa butter. Numbers 1 and 2 were fed with egg yolk to add 742 mg cholesterol per day to the diets, or without egg yolks. The polyunsaturated to saturated fatty acid ratio of the mixtures varied from 1.6 to 0.1. The "7 treatments, 4 replication" design did not permit each group to receive all diet fats, but each diet was fed in each of four periods of 5 weeks each. Therefore, "statistical adjustments in the calculated treatment means were necessary." However, each group did receive a different fat each period. Blood samples were taken twice a week.

The P/S ratios had no effect on serum cholesterol but the egg yolk raised it from 193 to 217 mg/dl, and from 188 to 215 mg/dl when added to fat mixtures #1 and #2, respectively. In spite of the incomplete Latin square design, the authors felt that the cholesterol values obtained for each fat over
the entire study are comparable, thus the cocoa butter was neutral.

E) Conner et al. (38) have reported a well-controlled study with healthy prison volunteers in a metabolic ward. Mixtures of fats were fed at approximately 110 g/day (ca. 40% of calories) with or without added cholesterol (ca. 725 mg). When fats of 2:3 and 1:4 P/S ratios were fed with cholesterol, the serum cholesterol levels after 4 weeks were 213 mg/dl and 202 mg/dl, respectively, and without cholesterol 175 mg/dl and 174 mg/dl. The saturated fat was supplied mainly by cocoa butter.

Not only does this study demonstrate that the saturated cocoa butter is not hypercholesteremic, but also that the P/S ratio is not a phenomenon, as all the differences in serum cholesterol can be attributed to the cholesterol content of the diet, supplied as egg yolk and suet.

F) Keys, Anderson and Grande (32) have attempted to explain discrepancies in their formula,

\[ \Delta \text{cholesterol} = 1.35(2\Delta S - \Delta P) + 1.5 Z \]

where S and P are percentages of calories produced by glycerides of saturated and polyunsaturated fatty acids of the diet, and Z is the number of milligrams cholesterol per 1,000 kcal when cocoa butter is included in the diet. In this formula all saturated acids have the same weight. They examined the results of their own experiments and those of others, especially with cocoa butter, concluding that “all available data are consistent with the theory that stearic acid in the diet is not cholesterol-promoting” and, in essence, that the relative lack of response of serum cholesterol to cocoa butter ingestion is due to its high (approximately 35%) stearic acid content. The authors then limit the S in their formula to the C12, C14, and C16 carbon acids, giving equal weight to each. As will be reviewed below, experiments with those acids have led to conflicting results, even from the same laboratories.

G) Connor et al. (39) tested the hypothesis that the mechanism of action of polyunsaturated oils is related to increased steroid excretion. In the design of this study cocoa butter was contrasted with corn oil. The latter was “redistilled in order to remove enough of its high content of plant sterols so that the plant sterol content of the corn oil and cocoa butter were approximately equivalent.”

The subjects were given the cocoa butter as 40% of the calories of a liquid formula diet for 3 weeks, the same amount of corn oil for 3 weeks, and the cocoa butter again for a final 3 weeks. The formulas were cholesterol-free, but the plant sterols ingested totaled between 300 and 411 mg/day, “dependent upon the kind of dietary fat.” The latter statement makes one wonder at the quantitative meaning of the word “approximately” used in the explanation of the plant sterols in the oils, and whether one oil supplies the 300 mg and the other the 411 mg. That this point is critical will become evident below.

The average serum cholesterol concentrations of the six men on the three diets (two assays made 3 days apart during the 3rd week of each of the three dietary periods) were: cocoa butter 222 ± 13 mg/dl, corn oil 177 ± 14 mg/dl, and cocoa butter 225 mg/dl, respectively. Thus, there is an unchallengeable difference between the levels on the cocoa butter and corn oil diets. However, it should be noted that on mixed natural foods the value was 277 ± 26 mg/dl, 52 to 55 mg/dl higher than on cocoa butter.

The authors make no attempt to speculate on the contribution of cocoa butter to the difference between the responses to the two oils, but give the impression that they consider it all to be due to the corn oil. They must, therefore, have assumed that the cocoa butter was neutral.

One can conclude that the high value on normal diets is due to diet cholesterol and the low value on the corn oil diet, to either its polyunsaturated acids or to its phytosterols, or to both. The medium response of cocoa butter is probably the same response one would obtain from the use of a neutral oil or even a low fat diet. Certainly no hypercholesteremic effect of cocoa butter is demonstrated.

The low serum cholesterol that accompanied the ingestion of corn oil, relative to cocoa butter, would on first glance appear to be an effect of its polyunsaturated acids be-
causation the sterol contents of the two oils were said to be “approximately equivalent.” However, one oil apparently had 30% more sterol than the other, and what may be more important, the nature of the sterols differed. The sterols in corn oil are γ-sitosterol, β-sitosterol, and stigmasterol in ratios of 9.5:88.8:1.0, with an unknown contributing 4.9 parts (40). The differences in the β-sitosterol to stigmasterol ratio of approximately 90:1 and 62:24 in the two oils cannot be lightly brushed aside. It has been reported that campesterol is the most active hypocholesteremic sterol in soybean and wheat sterols in the chick (41), whereas in algal sterols, fucoxsterol is more effective than sargassum sterols (42). Campesterol has been found to be better absorbed than sitosterol (43).

The importance of the plant sterols, rather than the polyunsaturated fatty acids as the factor in corn oil responsible for its hypocholesteremic effects, will not be reviewed here. It might be noted that there was a greater fecal steroid excretion on the corn oil diet, due mainly to lithocholic and deoxycholic acids, indicating interference with reabsorption of the mono- and dihydroxy bile acids (a greater degree of dehydroxylation of cholic acid) on the polyunsaturated fat diet. One must give credence to the possibility that the difference in response to the two oils is a reflection of the amounts and kinds of plant sterols.

Whatever the explanation for the increased amount of steroid excretion on the corn oil diet, it was not proved that the saturated cocoa butter was hypocholesteremic, but only that it was neutral.

H) In a comparison of the serum cholesterol response of a number of men to stearic acid ingestion, Grande et al. (34) offered 32 men, 40 to 65 years of age, four diets each containing 775 kcal/day: 1) a mixture of 94% cocoa butter and 6% safflower oil (94CB); 2) an imitation cocoa butter made of palm oil, totally hydrogenated soybean oil, and olive oil, having the same fatty acid composition as 94CB (ICB); 3) natural palm oil (PO); and 4) imitation palm oil made of totally hydrogenated soybean oil and olive oil (IPO). The basal diet “comprised foods usually found in American diets,” estimated to contain approximately 245 mg cholesterol and 20 g fat per day.

The men were divided into four groups of seven or eight men each, “matched as to age, relative body weight, and serum cholesterol concentration.” The four diets were given in staggered rotation to each group for 18 days. Fasting venous blood was drawn at the end of each 18-day period.

The fatty acid composition of the supplements differed significantly only in their palmitic acid contents. These values and the mean serum cholesterol concentrations at the end of each period are given as reported in Table 1.

Attempts to obtain trustworthy stool samples for determination of fatty acid absorption were inadequate for purposes of comparing groups and different supplements.

The data were examined by analysis of variance. It was concluded that “Diet PO produced significantly higher serum cholesterol levels than diets ICB and 94CB, the differences being, respectively, 18 and 14 mg/dl.” The authors also might have pointed out in favor of their position that by their criterion (a difference of 9 mg/dl) diet PO produced higher levels than IPO. Likewise, diet 94CB produced serum cholesterol significantly higher (10 mg/dl) than diet IPO. On the other hand, there was no significant

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Percent palmitic and stearic acid composition of supplements and group mean serum cholesterol concentrations, milligrams/deciliter</th>
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<tbody>
<tr>
<td>Diets*</td>
<td>94CB</td>
</tr>
<tr>
<td>Tripalmitin</td>
<td>27</td>
</tr>
<tr>
<td>Tristearin</td>
<td>31</td>
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<td>Groups</td>
<td></td>
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<tr>
<td>W</td>
<td>209</td>
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<td>196</td>
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<td>Y</td>
<td>200</td>
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<td>Z</td>
<td>208</td>
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<tr>
<td>Mean ± SD</td>
<td>203</td>
</tr>
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<td></td>
<td>± 29</td>
</tr>
</tbody>
</table>

*CB = cocoa butter; ICB = imitation cocoa butter; PO = natural palm oil; IPO = imitation palm oil.
serum cholesterol difference between diets IPO and ICB, in which there was a considerable difference in amounts of palmitate and stearate.

The data can be looked at differently and more pragmatically by arranging them in increasing order of the average serum cholesterol concentrations they produced: IPO 193, ICB 199, 94CB 203, and PO 217 mg/dl. Considering the normal variations first, then secondly, that the subjects were on the respective diets for only 18 days, and thirdly, that only one assay was made per period, it is reasonable to conclude that the serum cholesterol values of 193, 199, and 203 do not differ at all even by the criterion of 9 mg/dl, and that only 217 may be high. Therefore, all the lengthy and involved rationalizations of this paper are meaningless. Because the degrees of absorption of the different fats were not determined, one is justified in assuming that the degrees of absorption of the supplements varied inversely with the amounts of tristearin (44), which was low only in the PO supplement, 7% as compared with 30 to 38%.

Also, as it is well established that cholesterol absorption is closely related to fat absorption, the results of this study can be explained simply by relating serum cholesterol concentrations to the amounts of cholesterol absorbed, which in turn, are dependent on the amounts of fat absorbed. Obviously, more PO must have been absorbed than any of the other fats or mixtures.

Nevertheless, the reviewer agrees with the conclusion the authors reached after much rationalization: cocoa butter and stearic acid are neutral with respect to their effect on serum cholesterol concentration. However, because of the presence of cholesterol in the diet and the interrelated absorption effects noted above, he finds questionable the authors’ interpretation that “isocaloric replacement of carbohydrate by 31 g of palmitic acid will cause an increase of serum cholesterol concentration of 24 mg/dl” in the sense meant by the author, i.e., that the palmitic acid, per se, was hypercholesteremic. Paradoxically, these authors have elsewhere reported that stearic acid in cocoa butter counteracts the effect of its palmitic acid (45).

In addition to the above criticisms, any conclusion from this study is suspect due to the short period of 18 days during which the subjects were maintained on the respective supplements. No effort was made to establish confidence that a steady state or plateau was reached, and no person was tested for the entire period of 72 days on one diet to establish variation with time. In short, there was no control.

Cocoa butter is thus exonerated as being hypercholesteremic, even by the supporters of the saturated fat theory. This cannot be lightly dismissed. If saturated fat in the diet does raise serum cholesterol concentration, cocoa butter should certainly do so. But it doesn’t, and the fact makes one pause and look more carefully at those data which purport to demonstrate the theory.

Coconut oil

In the early days in the development of the saturated fat theory it was thought that any fat or oil causes high serum cholesterol concentration (46). For several years during this period, fats and oils fed were not identified.

This idea was quickly replaced by the animal fat versus vegetable oil hypothesis (47, 48) until evidence was adduced placing coconut oil in the animal group with regard to its effect on serum cholesterol (49, 50). Coconut oil and its hydrogenated product were easy to obtain, they contain no cholesterol, and were thus favorite fats to use in saturated fat diet–serum cholesterol studies. This was indeed unfortunate because it is a most unusual fat containing approximately 45% lauric acid, which is suspected of being toxic when fed at high levels, especially in choline- (51, 52) and vitamin B₆- (53, 54) deficient diets. Furthermore, coconut oil and especially the hydrogenated product are low enough in linoleic acid that marginal or frank linoleic acid deficiency can develop with its use.

Unlike cocoa butter, the consensus has been that coconut oil is hypercholesteremic, the data from studies with coconut oil form-
ing the backbone of the saturated fat theory. For practical reasons, because coconut oil is only a specialty diet item and thus consumed in minute amounts by any one individual, except perhaps by persons who drink filled milk, it would be far more important to consider the effects of hydrogenated cottonseed, corn, and soybean oils. Unfortunately, there are few data on these more frequently used products. Stripped animal fats such as butter, lard, or tallow would also be better test fats for assessing the effects of saturated animal fats, but almost no data are available except a little with stripped butter, which will be examined below.

Because of the key role coconut oil studies have played in the saturated fat hypothesis, more papers will be analyzed here than those on other fats and oils. It must be pointed out that not all studies with coconut oil have found it to be hypercholesteremic.

A) Kinsell et al. (55) reported on one subject fed an 1,800-kcal diet including, in turn, about 800 or 900 kcal of: 1) a synthetic triglyceride containing 74% oleic acid, 19% palmitic acid, 5% stearic acid, and 2% linoleic acid; 2) the synthetic fat plus 10 g crystalline cholesterol/d; and 3) coconut oil. Although there was only one subject, this paper is reviewed because it was most influential in establishing the concept that diet cholesterol is not a factor in serum cholesterol levels but that saturated fat is, and coconut oil is representative of saturated fat.

On the synthetic fat diet, the total serum cholesterol concentration rose sharply from the initial level on a free diet of about 175 mg/dl to nearly 235 mg/dl in 3 days, and then dropped down to 110 mg/dl and back to almost 125 mg/dl in 20 days at the time cholesterol was added to the diet. After the introduction of cholesterol as an emulsion into the synthetic fat formula, the total serum cholesterol slowly rose from the 125 mg/dl level to approximately 150 mg/dl in 10 days but was back to 110 mg/dl at the time the cholesterol was removed 5 days later. Institution of the coconut oil diet elicited a rapid rise in serum cholesterol concentration back to 175 mg/dl in approximately 10 days. No values are given, all the above figures being estimates from a highly reduced, difficult-to-read figure.

Both the absolute values and the fluctuations of serum cholesterol concentrations are suspect. On the normal diet, the serum cholesterol varied from 175 to almost 250 mg/dl. The reduction to 110 mg on the institution of the synthetic triglyceride is unconvincing. This was only for 1 day, it is unreasonably low, there was no plant sterol in the triglyceride, and the linoleic acid level was too low (2%) even for polyunsaturated enthusiasts. Either the subject was a poor choice (he had multiple fractures that could effect fluctuations in serum lipid levels) or the serum cholesterol assays were in error, or both.

No confidence can be placed in this study as proof of the hypercholesteremic property of coconut oil. It should also be noted that there was no diet cholesterol effect, probably because it was administered in crystalline form. This was one of the studies that led to the conviction that diet cholesterol was not a factor in high serum cholesterol.

B) In another study from a laboratory of primary importance in the development of the saturated fat theory, 12 schizophrenic men, 32 to 62 years of age, were placed for 4 weeks on a "house diet" and then for 4 weeks on a "low" (40 g/day) fat diet (56). Carbohydrates, equal to 40% of the calories of the low fat diet (not described), were then replaced by 100 g cottonseed oil for six of the men and by 100 g coconut oil for the other six. After 4 more weeks, the latter two oils were reversed for the two groups for an additional 4 weeks. Serum cholesterol determinations were made at the end of each 4-week period, and the average differences produced by the two diets were compared.

The serum cholesterol concentrations are not given. The mean differences and the standard errors between the coconut oil and house diet serum cholesterol concentrations in the combined data from both groups was $+11 \pm 2.8$ mg/dl, and between the coconut oil and cottonseed oil diets it was $+46 \pm 6.8$ mg/dl.

The skimpy and conflicting data are frustratingly difficult to assess. Forty grams of
fat per day (9 to 12% of the diet) is not a low fat diet. Furthermore, it was claimed that the two groups of six men consumed an average of 308 mg cholesterol/day in the cottonseed oil diet and 305 mg/day of cholesterol on the coconut oil diet. But this would mean that the fat contained 750 mg cholesterol/100 g, with butter constituting only approximately 25% of the total fat consumed. The 300 mg figure for milligrams of cholesterol consumed per day has to be in error unless eggs were eaten.

In addition, the confusion between the two statements describing the amount of test oil in the diet, the lack of information on the composition of the "low fat" diet, and the dearth of data on absolute serum cholesterol concentrations add nothing to the confidence we can place in the conclusion from this report, that the substitution of coconut oil for cottonseed oil results in 56% higher serum cholesterol concentration.

One is impressed by the statement, "Unfortunately, there was poor agreement between the two low fat levels, and the variability of the individuals was great."

C) The landmark paper by Ahrens et al. (15) analyzed above for its data with patients tested with hydrogenated oils and with cocoa butter, also contains data on the response to coconut oil. The conditions under which the coconut oil was used are similar to those described for the hydrogenated oils, including the preconditioning with corn oil (q.v.).

Three of the patients were given coconut oil. One (#9) was designated only as hypercholesteremic. The other two (#28 and #38) were simply designated as having atherosclerotic heart disease, #28 with myocardial infarction.

The serum cholesterol concentrations of these patients (9, 28, and 38) on ad libitum diets were 430, 267, and 186 or 170 mg/dl (two determinations), respectively. After 3 weeks on the corn oil formula diet, the serum cholesterol concentrations "stabilized" at 263, 156, and 105 mg/dl, respectively, and after 3 or 4 weeks on coconut oil, they were 367, 213, and 186 mg/dl. After an additional period on the corn oil diet, the values again fell to 286 and 156 in patients 9 and 28. (Patient 38 was given safflower oil at this point and his serum cholesterol level became 111 mg/dl.) The higher values produced by the coconut oil above those produced for the first corn oil trial were thus 104, 57, and 81 mg/dl, and by the second 81, 57, and 75 mg/dl.

These conditions are subject to the criticism that they test the differences in cholesterol concentrations effected by corn oil versus coconut oil and cannot be ascribed to the coconut oil. All the differences could be due to the constituents of the corn oil.

Note that the steady-state levels after coconut oil ingestion were still well below those which two of the three patients experienced on their ad libitum diets. Thus, the coconut effect was a neutral one between the cholesterol-containing ad libitum diet and the sitosterol-containing corn oil diet. It is not unlikely that olive oil or fat-free diets would have given results similar to those of the coconut oil diet. The experiment, therefore, does not demonstrate a hypercholesteremic response to coconut oil.

D) Malmos and Wigand (18) fed analogs of cheese and ice cream in which the butterfat was replaced by certain fats and oils to be tested. The test oils were also added to mashed potatoes and porridge and used as salad dressings and frying oils.

Serum cholesterol concentrations were determined twice a week while the subjects remained on the test diets for 3 to 6 weeks. The authors concluded that hydrogenated coconut oil does not lower serum cholesterol concentration in comparison to "free" diets, but they made no observation as to its capacity to raise it to high levels. Their data show that in every case the hydrogenated coconut oil diet followed the "free" diet and that the serum cholesterol concentrations stayed at the level of the "free" diet. When corn oil or other polyunsaturated vegetable oils replaced the coconut oil, the serum cholesterol concentrations promptly dropped, even though the coconut oil substitution was only partial, i.e., two-thirds coconut oil, one-third corn oil.

Significantly to the saturated fat theory, the authors ascribed the lack of response to the coconut oil following the "free" diet to
the lack of linoleic acid, not to a hypercholesteremic quality of saturated acids.

The compositions of the “free” diets are unknown, but the starting serum cholesterol concentrations varied from nearly 200 mg/dl to over 400 mg/dl, and they did not change significantly in most trials after institution of the coconut oil diets. However, it must be noted that hydrogenated coconut oil diets, with two exceptions, were maintained for only 1 week following the free diet. The other oils followed the coconut oil and lasted several weeks. In one of the two cases, the serum cholesterol did drop from 320 mg/dl to 280 mg/dl during the coconut oil period. Therefore, there is no evidence here for a hypercholesteremic response to coconut oil, but as is so often the case, all the changes can be ascribed to removal of the cholesterol from the diet and the addition of sitosterol-containing oils.

In more recent studies with the cynomolgus monkeys, the same authors (57) report “only a slight to moderate increase of serum cholesterol” with hydrogenated coconut oil. But in this study as before, the coconut oil diet followed a mixed diet, the composition of which is not given.

E) In a study by Okey et al. (58), the response of plasma cholesterol and other plasma lipids to safflower and coconut oil ingestion as 80% of the fat in the ration of 30 male prisoners was studied. Each man was his own control, the safflower and coconut oil diets being sandwiched between periods of 4 to 5 weeks of regular prison diet.

The total plasma cholesterol concentrations for three periods on prison fare were 201 ± 1, 171 ± 7, and 200 ± 7 mg/dl. The values on the safflower and coconut oil diets were 165 and 203 mg/dl, respectively.

Lack of confidence arises from the authors’ conclusions that there are significant differences between the controls, thus making the values after both safflower oil and coconut oil diets significantly different from one control but not from the other. It is obvious that one can draw his own conclusion by selection of which control he wishes to use.

The failure of the serum cholesterol concentration to return to the starting level during the 2nd control period is disconcerting. All diets contained nearly 400 mg of cholesterol per 100 g fat, which were supplied in the experimental diet in “wafers containing weighed amounts of cholesterol dissolved in the experimental fats.”

One possible explanation for the high serum cholesterol gain on the coconut oil diet may lie in the fact that there was a mean weight gain of 2.5 lb per man during the month on the coconut oil diet versus 1 lb during the safflower oil diet, no mention being made of the others. Could there have been a loss of weight during the 2nd control period?

The difficulties with this study are typical of the literature supporting the saturated fat theory. The difference between serum cholesterol concentrations of 165 mg/dl and 203 mg/dl is large and persuasive, yet after careful study of all the factors involved, one must conclude that no conclusion can be reached from the data, vis-à-vis a hypercholesteremic quality of coconut oil.

F) Another report in which the authors interpreted their findings to indicate that coconut oil ingestion results in high serum cholesterol concentrations, but which can be interpreted to the contrary, has come from a Minnesota laboratory (59). The lack of clear-cut results can be attributed to the relatively low percent of coconut oil in the test diet and to the high level of cholesterol in the comparison diet made up of oleo-stock. This again points out the need for a common reference diet. Oleo-stock is the liquid fat remaining after crystallization of stearin from beef tallow and might be expected to contain much of the tallow cholesterol. The coconut oil in the diet (given to schizophrenic men ages 38 to 68, classified neither according to serum cholesterol levels at the start nor to some standard diet) supplied only approximately 15% of the 3,085 kcal a day consumed, olive oil and fat from the basal diet supplying almost 26%. This diet was used in a “switchback” type of experiment with a diet of 98% oleo-stock and 2% safflower seed oil. The test trial periods were 3 weeks. After 3 weeks on the oleo-stock diet, the average value from 14 men was 197 mg/dl, and after the subsequent coconut oil diet it was 186 mg/dl. In the
reverse switchback, the response to the coconut oil was an average of 211 mg/dl and to the oleo stock diet 184 mg/dl.

In calculation of the differences between the two diets, the values are "corrected for time trend by increasing each value in period 2 by 19," following a quoted procedure (60). Following such calculations, the authors concluded that the coconut oil produced a serum cholesterol concentration higher by 8 mg/dl than the "oleo stock diet." As normal variations are greater than this, a hypercholesteremic effect of coconut oil was not shown. However, the authors claimed it to be a significant difference, a demonstration of hypercholesteremic response to coconut oil, and further proof of the saturated fat theory.

G) In a test of the validity of the P/S postulate, Gunning et al. (61) fed 2 patients, C and A, coconut oil at 45 and 40% of the calories, respectively, in liquid form for at least 3 weeks, followed by other fats and oils at 52% of the calories.

Patient C had "controlled" hypothyroidism. His serum cholesterol concentration held at 319 ± 7.3 mg/dl during the 3 weeks on the coconut oil formula. This was followed in order by butter (256 ± 7.5 mg/dl), beef fat (237 ± 4.6 mg/dl), chicken fat (216 ± 3.6 mg/dl), a mixture of vegetable oils (209 ± 10.2 mg/dl) and safflower oil (186 ± 3.2 mg/dl).

Patient A had atherosclerosis (sic). Following a normal diet, the oils were used at 40% of the calories in his formulas, and the corresponding serum cholesterol concentrations in milligrams/decilitre were in turn: coconut 331 ± 10.7, safflower 222 ± 6.1, cottonseed 223 ± 3.8, soybean 206 ± 4.1, corn 197 ± 4.2, and avocado 177 ± 4.1.

H) One normal and three subjects with familial hypercholesteremia and hyperglyc eridemia were utilized to assay biliary excretion as an explanation for the purported reduction in serum cholesterol effect by polyunsaturated fats (64). Only the effects of the diets on serum cholesterol will be examined here. Six different diet triglyceride mixtures were used. They were "synthetic" corn and coconut oils made up of "pure" fatty acids, triolein, "palmitic-oleic glycerides," and commercial coconut and corn oils. The fat mixtures were given as 40% of liquid formulas. The corn oil had 975 mg plant sterols per 100 ml and the coconut oil 68. The synthetic mixtures had none.

The subjects were hospitalized in a metabolic ward for the 3 to 6 months required for the studies. They were said to have been "stabilized" on the first oil on which they were tested before beginning the second. The criteria for "stabilization" are not given, unfortunately, although in a previous study from the same laboratory (15), stabilization was said to have been accomplished when the standard deviation of serum cholesterol changed not more than ± 20 mg/dl over an unstated period of time.

Patient RC (a hypercholesteremic whose serum cholesterol level on a normal diet was not given) was fed trilinolenin, the mean serum cholesterol concentration being 286 ± 17.6 mg/dl during 4 weeks. After a 1-week transition period on "palmitic-oleic glycerides," the mean value given for the stabilized period on this synthetic glyceride is 340 ± 20 mg/dl. In contrast to the mean values, the last values given on the trilinolenin and "palmitic-oleic glyceride" diets are approximately 290 mg and 300 mg, respectively. Thus, there was no difference at the end of 4 weeks on each of the oils. From the graph it appears that there was a temporary rise in serum cholesterol brought on by the change to the "palmitic-oleic glycerides," but that it was returning to the trilinolenin level toward the end of the period. In light of the trends shown while the patients were on the two oils, and the terminal values, the "mean" values are meaningless.

Patient JG ("hypercholesteremic") was stabilized with the synthetic coconut oil. The serum cholesterol levels given in the chart varied from approximately 575 to 650 mg/dl. When stabilized after a 2-week transition period on synthetic corn oil, the value dropped to between 425 and 475 mg/dl. The mean values given in the table are 615 ± 15.2 mg/dl on the synthetic coconut oil and 462 ± 8.7 on the synthetic corn oil. This represents a clear-cut difference between the two oils, but there is no way of knowing whether the "coconut oil" made it
high or the "corn oil" made it low. But what is more important, the patient was probably still reacting to the removal of his normal cholesterol-containing diet, a prominent phenomenon of some hypercholesteremias. Without controls or a neutral diet, or reversing the order of the oils, one can draw no conclusions with regard to a hypercholesteremic property of coconut oil.

The standard deviations of only ± 15.2 and ± 8.7 for mean serum cholesterol concentrations of 615 and 462 mg/dl lack verisimilitude.

Subject J. McG was used twice. In the first test, the two oils were "synthetic coconut oil" and "synthetic corn oil" given in that order. The serum cholesterol levels on the "synthetic coconut oil" cannot be said to have been stabilized, as at the end of each of the last 3 weeks on that oil, the serum cholesterol concentrations were rapidly dropping, being approximately 180, 160, and 150 mg/dl, respectively. It continued downward on the same slope (graph given) to almost 130 mg/dl during the 1-week transition to "synthetic corn oil." Serum cholesterol stayed at that level during the synthetic corn oil feeding period, yet the mean values given in the table for the two periods are 165 ± 11.0 mg/dl and 122 ± 5.0 mg/dl, respectively! Quite obviously this subject was still adjusting to the removal of his normal cholesterol-containing diet, and no conclusions can be drawn relative to the cholesteremic properties of the oils. Nevertheless, a constant decrease from 180 to 150 should be evidence that coconut oil is not hypercholesteremic.

In the second trial with the normal J. McG, commercial corn oil preceded commercial coconut oil, with a 2-week transition period between them. (It should be made clear that the so-called "transition period" was purely arbitrary, being the period on the second diet during which the serum cholesterol was said to have become stabilized.) The mean values for the stabilized periods as given in the table are 137 ± 3.5 mg/dl and 180 ± 9.6 mg/dl for the corn and coconut oils, respectively. Although the values on the coconut oil diet fluctuated approximately 30 mg/dl, one can accept the conclusion that there is a difference of 43 mg/dl between the effects of the two oils. But one cannot know whether it was due to the effects of one or the other, or both. Also, these were natural oils. The corn oil contained 975 mg sterol per 100 g and the coconut oil only 68. It is quite possible that the difference between these oils is an effect of the quantitative differences in their constituent phytosterols.

MS (hypercholesteremic) also was assayed for his responses to the commercial oils, coconut oil following corn oil. Although this patient was classified as a hypercholesteremic, his starting serum cholesterol concentration on the corn oil formula was only 119 ± 4.3 mg/dl, which rose to 190 ± 2.8 at stabilization on corn oil. Thus, his serum cholesterol concentration was actually rising while he was on the corn oil diet. This was accelerated by substitution of the coconut oil, a response which could have been due to the removal of the 1% phytosterols with the corn oil. If this patient was a hypercholesteremic as claimed, the coconut oil cannot be held responsible for elevating his serum cholesterol concentration after removal of plant sterols from his diet. One should expect that it would have risen if all oil had been removed from his diet. It is unfortunate that his serum cholesterol concentration on a normal diet was not given.

Thus, results of all five trials are clouded by inadequate length of time of adjustment, especially from normal to coconut oil diets, and the great differences in the amounts of plant sterols in the natural oils. Certainly the data do not demonstrate that coconut oil is hypercholesteremic, but rather that it is neutral.

I) The following study is strongly contrary to the concept that coconut oil is hypercholesteremic, although at first blush it appears to support it (65). In a study designed to test the effects of high and low sucrose and high sucrose–lactose mixtures and their interactions with the nature of diet fat, 38% of the calories as olive, safflower, and coconut oils were fed to schizophrenic men. Two test groups consisted of nine men each, each group being "subjected" to consecutive periods of 12 weeks on each carbohydrate diet, divided into three 4-week periods on each of
the three oils. The only interaction between fat and type of carbohydrate was a high response to the combination of lactose and coconut oil. The average serum cholesterol values on the oils and standard deviations were: olive oil diet 204 ± 30; safflower oil diet 172 ± 22, and coconut oil diet 252 ± 30 mg/dl.

The relatively high serum cholesterol value on the coconut oil can be attributed to the lasting effect of the previous cholesterol-containing normal diet. The continuing drop in serum cholesterol values on successive formulas of polyunsaturated oils was attributed by the authors "to the fact that after the initial fall, with continued intake of such fats there is a very slow continuing decrease." As was pointed out in the analysis of the hydrogenated corn oil study by Ahrens et al. (15), it would have been a far better test to have given the oils in the order of increasing saturation and thus control the effect of removal of cholesterol from the diet. This factor is repeatedly ignored. Ideally, if the oils are fed in the order of decreasing saturation, the order after the last oil is fed should be reversed and the test repeated. Should the serum cholesterol concentration then climb, the point could be proved, or at least should deserve more credence.

This study exemplifies the error in the common practice of using coconut oil as the first oil after the ad libitum diet. In addition, this report fails to record the serum cholesterol concentration on the ad libitum diet, making any definitive conclusions vis-à-vis the effect of coconut oil impossible.

The P/S ratio hypothesis of Jolliffe et al. (62) and the 2S-P theory of Keys and coworkers (31) were found not to fit the data.

The patients used in this study are examples of individuals who may benefit from the use of a low cholesterol diet because removing cholesterol-containing fat from the diet resulted in reduction of serum cholesterol from high to low risk levels. In all likelihood they were patients with type II hyperlipoproteinemia, so that one cannot extrapolate this to saturated fat per se, nor to all persons. Hypothyroid patients exhibit type II hyperlipoproteinemia (63).

As given here, the high serum cholesterol response to coconut oil ingestion invites credence. The olive oil could have represented a base-line oil if the experimental design had not been defective or if more information had been given. There are, however, some disturbing factors. The serum cholesterol concentrations of the subjects on the "typical American diet" (on which they were maintained for 1 month before and after the tests) are not given, nor is the order in which the oils were given. Thus, if the coconut oil was given first, followed by olive and safflower oils, the high serum cholesterol values on coconut oil could be explained by inadequate time for adaptation from the normal cholesterol-containing diet and the hypocholesteremic effect of safflower oil phytosterols.

This is an excellent example of how data on the subject can be misleading, even to the most astute investigators themselves.

J) This is a 5-year study of 100 men between the ages of 20 and 50 years with electrocardiographically confirmed myocardial infarctions (66). The objective was to contrast the effects of two 28% fat diets, one containing a 1:1 mixture of corn and safflower oils, and the other a 1:1 mixture of coconut and peanut oils. "Certain dairy products, rich desserts and pastries, certain fried foods, and fatty meats" were eliminated from the diets.

Because of some question of adherence to the diet during the first 12 months, a prepared, frozen food regimen was offered and closer controls maintained over the subjects during the final 4 years. After 5 years, the serum cholesterol values, as shown in Table 2, were found.

Thus, there was no difference in serum cholesterol concentration after 5 years of ingestion of 28% of the total calories as coco-

TABLE 2
Serum cholesterol concentration, in milligrams per deciliter

<table>
<thead>
<tr>
<th></th>
<th>Corn-safflower</th>
<th>Coconut-peanut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-experimental</td>
<td>259 ± 5.6</td>
<td>262 ± 4.7</td>
</tr>
<tr>
<td>Final</td>
<td>234 ± 5.6</td>
<td>243 ± 4.7</td>
</tr>
<tr>
<td>Change</td>
<td>-25</td>
<td>-19</td>
</tr>
</tbody>
</table>

* Standard deviation.
nut and peanut oils as compared with the same amount of corn oil–safflower oil mixture. The linoleic acid content of the coconut oil diet was 3.3% and of the corn oil diet was 14.1%. The respective P/S ratios of the diets were 1:3 and 3:1. This study is strongly contrary to the concept that coconut oil is hypercholesteremic.

K) Spritz and Mishkel (67) fed four patients formula diets in which coconut, safflower, and corn oils constituted 40% of the calories. Subjects 1 and 2 were normocholesteremic and 3 and 4 were hypercholesteremic.

The coconut oil formula was given to the four patients for 21, 28, 28, and 28 days, respectively. No value for serum cholesterol level is reported until the 20th day, but the values at the end of the periods were 210 ± 13, 265 ± 19, 400 ± 19, and 390 ± 10 mg/dl. The four subjects then received the polyunsaturated oils; number 3 received corn oil, the other three safflower oil.

Values are shown on a chart from the 20th day on coconut oil to the end of the polyunsaturated vegetable oil ingestion periods of 21 or 28 days. A sharp drop occurred during the first 8 or 10 days on the polyunsaturated fat diet, and then a plateau or slight rise was achieved the last 10 days, at which time the values were 145 ± 18, 100 ± 12, 352 ± 24, and 225 ± 19 mg/dl. It is impossible to interpret this data for any conclusions concerning coconut oil because:

a) With no neutral reference diet, the sharp drop completed in 8 to 10 days after initiation of the polyunsaturated fat formulas can be ascribed either to an effect of the polyunsaturated fat, to their constituent phy-tosterols, or to the removal of coconut oil.

b) Without controls maintained on the normal diet (to which the change to coconut oil or a neutral oil formula could be compared during the entire period), one can argue that removal of the normal diet alone might have been responsible for the decrease in serum cholesterol concentration during the 8-week test period.

L) In an epidemiological type report (68) designed to study the effect of coconut oil on the fatty acid composition of adipose tissue of Maori Polynesians on the Island of Pukapuka (who consume 80% of the 7 g fat per day as coconut oil), serum cholesterol concentrations were also measured. The values were compared with similar Polynesians (Maori) living on a different island (Rarotonga), who consume a western-type diet containing small amounts of coconut oil but generous quantities of milk, eggs, and ice cream.

Although the serum cholesterol values are not given in this paper, they are contained in the New Zealand Special Report Series, No. 26 (69). The values range from 159 mg/dl to 186 mg/dl for males aged 20 through 69, and 172 to 201 mg/dl for females, who are clearly not hypercholesteremic.

The list of reports of the response to coconut oil could be extended, and many more were examined (36, 70–75). The reviewer has tried to select those which best represent the case against the oil, though some contrary reports are also included. The oversights pointed out in the analyses are typical and, of course, are due in large part to the difficulties of working with humans. Nevertheless, the data do not demonstrate that coconut oil specifically, nor saturated fat in general, cause high serum cholesterol concentrations.

In addition, coconut oil is not a proper substance to use in tests of hypercholesteremic activity of saturated fat because of its special composition. Even if some abnormal responses to large amounts were found, it could be attributed to some special action of its high lauric acid or to its low essential fatty acid content. Nevertheless, when included in balanced diets in reasonable amounts, no untoward effects could be truly demonstrated to have occurred.

Stripped butterfat

Fats and oils can be freed of their sterols, as well as other nonglyceride constituents by molecular distillation. Such products are said to be “stripped.” There are few studies with stripped animal fats, though a number of studies have been conducted with stripped vegetable oils to test the effect of plant sterols. As a test of the ability of animal triglycerides or fatty acids (exclusive of cholesterol) to
cause hypercholesteremia, more ideal materials would be stripped lard, beef or sheep tallow, or butter. However, the only significant studies with stripped fats are those of Beveridge and his group with stripped butter, one example of which should suffice.

In a study reported in 1957 (28), 38 students were given for 8 days a basal diet containing 60% of the calories as corn oil. They were then divided into five groups. Butter, or the various cuts of the molecularly distilled butter oil, replaced the corn oil for an additional 8 days, although as only 40% of the calories. The butter oil and the first two distillate cuts contained, respectively, 0.146%, 1.60%, 0.115%, and the last two cuts contained traces of Lieberman-Burchard positive material.

The serum cholesterol concentrations were produced after 8 days by the butter oil, and the four cuts were almost 190, 220, 170, 170, and 160 mg/dl, respectively. However, because the values in each group varied significantly after 8 days on the corn oil, the percentage increase for each group was calculated and found to be nearly 41, 72, 29, 51, and 40, respectively.

The plasma cholesterol values for the five groups on the corn oil diet varied from approximately 110 to 140 mg/dl, the variation indicating either that the groups were not properly selected, or that the 8-day reference period was not long enough to stabilize plasma cholesterol. However, the differences between the levels reached on the low cholesterol cuts (160 to 175 mg/dl) and the high cholesterol cuts (220 mg/dl) indicate that the fatty acids in butterfat are not hypercholesteremic, but that the cholesterol in butter is mildly so in these young students.

The study, however, does not invite confidence. The Lieberman-Burchard values seem low for the amount of cholesterol in butter. The serum cholesterol concentrations on the 40% butter oil diet and after the various cuts tell a different story than the percentage increases. This is due, of course, to the variations in the groups after 8 days on corn oil. Butter oil, for example, produced no higher percentage increase than the cuts with only traces of the Lieberman-Burchard positive material, and even less than one.

Moreover, one cut with almost as much of the sterol as butter produced the lowest percentage increase.

Individual fatty acids

On the assumption that "saturated fat" is hypercholesteremic, several investigators have attempted to determine the relative cholesteremic power of the respective saturated acids. (The Minnesota group, by an analysis of the literature in which natural fats were used (32), have concluded that stearic acid is neutral and may even offset the effect of palmitic acid.) Other efforts have been made to pinpoint the responsible acids, or their relative hypercholesteremic power, using both natural and synthetic triglycerides. The analyses of a number of papers representative of this follow:

A) The paper by Ahrens et al. (15) contains a chart showing relationships between iodine values and serum cholesterol concentrations that has become quite familiar over the years. It appears to have ineradicably fixed in many minds the idea that the serum cholesterol concentration is inversely related to the iodine value of the ingested fat or oil, or ultimately, of their constituent fatty acids. The data were treated statistically, and first inspection of the chart appears to confirm the claimed relationship. But the data cannot stand close scrutiny, as exemplified by the response to lard.

There were four lard samples among the test fats. Their iodine values, the number of tests with each sample, and the percentage higher serum cholesterol value they produced other than that produced by corn oil are shown in Table 3, constructed from values in

<table>
<thead>
<tr>
<th>Iodine value</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>11 13</td>
</tr>
<tr>
<td>54</td>
<td>3 15 22 23 27 28</td>
</tr>
<tr>
<td>90</td>
<td>10 17</td>
</tr>
<tr>
<td>95</td>
<td>10</td>
</tr>
</tbody>
</table>
the chart. It is clear that there is no significant difference among the lards, recalling that each test was with a different person with no controls whatsoever.

If one removes the cholesterol-containing butter from the chart, the suggestive relationship between iodine value (IV) and cholesterol concentration disappears. (The special case of coconut oil was analyzed in that section.)

Two tests with cottonseed oil gave lower serum cholesterol concentrations than corn oil, and the highly saturated cocoa butter IV 37, in one test, gave a value of only 13% above corn oil, both comparisons of which are contrary to the saturated fat theory.

This work was done when cholesterol in fats was thought to have no effect on serum cholesterol concentration and no attention was paid to its possible influence in this study. The influence of plant sterols was also ignored.

B) Horlick and Craig (76) added corn oil, safflower oil, butterfat, ethyl stearate or an ethyl linoleate preparation (90% ethyl oleate and 5% ethyl stearate) to a 4% basal diet (containing 28 mg/100 ml cholesterol) consumed by male medical students and interns. Body weight, calorie and protein intake were kept constant except on the ethyl stearate diet.

The test oils and esters were given at 40% of the calories under varying conditions and for periods of only 1 to 3 weeks.

In one test, three subjects with serum cholesterol concentrations of approximately 200 mg/dl were given 40% of their calories for 1 week each as ethyl linoleate, safflower oil, and ethyl linoleate, again in that order. This was followed by ethyl stearate for 2 weeks. The diets also contained 600 mg crystalline cholesterol per day. It is possible that the cholesterol was not predissolved in oil, and therefore, would have been poorly absorbed. The serum cholesterol of all three subjects dropped steadily to the middle of the linoleate-cholesterol period to nearly 140 mg/dl, at which point it remained through the stearate feeding; returning to the 200-

mg/dl level 10 days after a normal diet was reinstituted.

The authors reasonably ascribe the response as simply the effect of removal of animal fat cholesterol from the diet upon substitution of the test diets and its subsequent return. The added crystalline cholesterol was judged to be relatively ineffective.

It was found that the stearate was not well absorbed as evidenced "from a loss in body weight and a change in the character and bulk of the stools."

In another trial with four subjects, each fed different test fats, a 4% fat regimen was fed for 2 weeks before the institution of fatty meals. These were added in increments of 10 g/day up to 70 g, at which time corn oil, ethyl linoleate, and ethyl stearate amounted to 23 to 26% of the calories. Butter was added in daily increments of 20 g up to 80 g/day or 26% of the calories. Butter was given for 12 days and the other "fats" for 21 days. Stools were analyzed for total fatty acids.

At the start of the 4% fat diet, the subjects to receive linoleate and corn oil had serum cholesterol concentrations approximating 150 mg/dl. These levels decreased to nearly 125 mg after 1 week and remained at that point until reinstitution of a normal diet.

The two subjects to receive butter or stearate had approximately 200 mg/dl serum cholesterol at the start of the 4% fat regimen, both becoming 160 mg/dl after 10 days. However, the value increased to almost 180 mg/dl on the day the butter diet was started and remained there during the entire test period.

The serum cholesterol concentration was at 160 mg/dl at the beginning of the stearate diet, dropping to approximately 140 mg after nearly 10 days. The stearate was said to have been 65 to 70% absorbed at this level (25.7% of calories) in the diet.

Although these data are presented by the authors in refutation of the hypercholesteremic hypothesis as far as stearate is concerned, and of the hypcholesteremic theory of polyunsaturated fats, it contains the same errors as the studies advanced to support them: there are too few subjects; the fats were not fed for adequate lengths of times; the failure of butter to increase serum cho-
lesterol is not reasonable; there are no controls for normal variation, et cetera.

C) In 1967, Thomasson et al. (77) gave volunteer male and female Trappists a liquid formula diet, 50% of the calories of which were either glyceryl trilaurin, olive oil, safflower oil, or various mixtures of these. There were 10 diets in all. In addition, they consumed bread, fruit, a vitamin mixture, and ferrous fumarate. The test period was 5 to 6 weeks, at the beginning and end of which blood samples were assayed for lipids. The pre-experimental plasma cholesterol levels varied between 86 and 222 mg/dl, in spite of the fact that the Trappists consumed dairy products. The means of the male and female subjects were 143 and 148 mg/dl, respectively.

The authors present a stereographic figure formed of regression planes to show the effects of the saturated, mono-, and dienoic fatty acids of the diet fat on plasma cholesterol concentration. However, beyond the statement, "the cholesterol level appeared to be strongly dependent on the type of dietary fat . . .," the authors do not interpret the figure with respect to this simple variable. Also, they do not give the plasma cholesterol values. It is frustrating to attempt to calculate the plasma cholesterol concentrations from tables giving the total plasma lipids per 100 dl, as the former are only given for the ends of the experimental periods. These cannot be compared because there was great variation between the groups in the total plasma lipids in the pre-experimental period. Thus, the total plasma lipids of the group which consumed trilaurin were 434 mg/dl, and those of the group receiving safflower oil measured 577 mg/dl. The fact that the total plasma lipids of the safflower oil group after 6 weeks consumption was essentially the same as the trilaurin group initially, 484 and 434 mg/dl, respectively, could indicate normal variations because there was no control and only one determination for each dietary condition. Furthermore, the percentages of cholesterol in the total lipids were remarkably constant in all groups at the end of the test period.

It is also disconcerting that the role of the plant sterols is ignored. One can reason that the lauric acid is neutral, that the olive oil values represent the effect of plant sterols, and that the response to safflower oil was due to the combined influence of plant sterols and linoleic acid. It appears to the reviewer that the data of this ambitious experiment are uninterpretable for purposes of comparing the responses to the individual fatty acids, though it was conducted for that purpose.

D) In a sequel to the above paper, a mixture of 90% trilaurin and 10% safflower oil was administered for 6 weeks to male Trappists as 20, 35, or 50% of liquid formula diets (78). The serum cholesterol remained between 195 and 205 mg/dl at all levels of diet fat, showing no effect of the saturated acid.

E) In other experiments from the same laboratory (private communication 1970), it was planned to compare the effects of synthetic triglycerides of pure fatty acids with natural oils.

Eight mixtures of 25 to 30% of either lauric, myristic, palmitic, or stearic acid and 70% of either oleic or linoleic acid were interesterified. These were compared with olive and safflower oils, each fat being given to Trappist monks 50 to 52 years of age as 50% of the calories of a liquid formula diet. Their serum cholesterol concentrations on their normal diet were 203 to 206 mg/dl. There was no cholesterol in the liquid formula diets nor in food supplements. After 6 weeks consumption of the four triglyceride diets (each containing one of the four saturated acids as 26 to 29% of the triglyceride acids, 68 to 71% as oleic, and 3 to 5% as linoleic), no significant difference was found in their effects. The authors noted that although the polyunsaturated to saturated ratio was 1:10 (so that an increase in serum cholesterol should have been expected), the value actually decreased significantly from the starting level. Whereas the authors thought of this as a refutation of the P/S theory, which is probably correct, a more logical explanation is the removal of cholesterol contained in the subjects' normal lacto-vegetarian diet and the neutral nature of the saturated acids.

F) The paper by Grande et al. (34), reviewed in the cocoa butter section, also con-
cerns the relative hypercholesteremic properties of stearic and palmitic acids. Because of the detailed analysis given there, it will suffice to say that the authors are probably correct in their conclusion that stearic acid has no such effect. However, the conclusion that 31 g of palmitic acid per day will increase serum cholesterol concentration by 24 mg/dl is a contradiction of another report from this laboratory (45) in which the lack of hypercholesteremic response to cocoa butter is interpreted to mean that stearic acid "may counteract the cholesterol-raising effect of palmitic acid," as the palmitic acid in the first mentioned study was always accompanied by stearic acid.

Because there was some cholesterol in all the diets used by this group, it is quite possible that what really was measured was the relative effects of the randomized and natural fat mixtures on the absorption of cholesterol.

G) Two reports from one laboratory, though 5 years apart, will be discussed here together (79, 80).

In the more recent of the two studies, the objective was "to test a series of dietary fats in which the specific saturated acids were varied independently of one another and of the monoenoic and polyunsaturated acids." In order to achieve these objectives, the following mixtures were transesterified: 1) 75% safflower oil; or 2) 75% olive oil with 25% trilaurin, trimyristin, tripalmitin, or hydrogenated soybean oil containing 85% stearic acid; 3) 60% medium-chain triglycerides (MCT) with 40% trimyristin, tripalmitin, or the hydrogenated soybean oil; and 4) 80% butterfat with 20% hydrogenated soybean oil.

Two further mixtures were prepared containing 80% of the MCT product with 20% of safflower oil or of olive oil.

Except for the diets containing butter, the cholesterol consumed each day was 306 mg, approximately that in one large egg. The butter-containing diets resulted in the intake of 442 or 552 mg/day.

The fats to be tested were incorporated into recipes for waffles, muffins, cakes, cookies, pie crusts, biscuits, salad dressings, and spreads for bread as 38% of the calories in a diet of normal foods such as fruit, vegetables, eggs, and meat.

Twenty-one schizophrenic males, ages 41 to 56 years, were utilized in two groups. Each group was given 15 different fat mixtures for 4-week periods, in addition to 4-week control periods on a normal diet between the test diets. Blood samples were taken on the 24th and 28th days of the 4-week periods and the two values averaged.

The absolute values of cholesterol concentrations were not given, only the differences between the averages of each test period and the averages of the control periods. The control diet supplied an average of 331 mg cholesterol per day.

The data were presented in two ways: 1) in a table giving the differences between the average serum values of total cholesterol, cholesterol in the \( \beta \)-lipoproteins, phospholipids, and triglycerides in the control periods; and 2) in graphs in which the data are "grouped into several series of test periods in which the long-chain saturated acids (C\(_{12}\) through C\(_{16}\)) are the principal variables to be compared."

Thus, the differences between the control diet and a) safflower oil diet, b) safflower oil with trilaurin, c) safflower oil with trimyristin, d) safflower oil with tripalmitin, and e) safflower oil with tristearin were presented in that order, although they were not ingested in that order.

The authors concluded that "although lauric and stearic acids were hypercholesterolemic under these conditions, they are less so than myristic and palmitic acids. The contrast in behavior of stearic acid to its almost complete ineffectiveness in a natural product (cocoa butter) suggests that... the position of a fatty acid on the glyceride molecule also influences its metabolism."

These authors, as did Grande et al. (34), failed to see that what they were really measuring was the effect of their diet fats on the absorption of the cholesterol. Clearly, this is why they find different responses to the same saturated fatty acids according to their position on the glyceride molecule. Palmitic acid in the 2-position is better absorbed than when in the 1- or 3-position, as has been determined in milk fats (81), and
cholesterol absorption is dependent upon fat absorption.

Although the authors point out in the 1965 paper (80) that phytosterols play a part in serum cholesterol levels and that this effect is partially dependent upon the composition of the dietary fat, no cognizance of this is taken in their experimental design or interpretation.

Thus, as late as 1970, the well-known interrelating roles of the plant sterols and the nature of the diet fat on cholesterol absorption were still ignored and different biochemical properties credited to respective fatty acids.

H) No review of the studies testing the saturated fat hypothesis can ignore the mathematical formulas of Keys and his group. It was first expressed in April 1957 (82) in the form of the least squares multiple regression equation:

\[ \Delta \text{chol} = 2.74(\Delta S) - 1.31(\Delta P) \]

where \( \Delta \text{chol} \) represents the change in serum cholesterol concentration in response to change \( \Delta S \) in diet saturated fatty acid (percent of total calories of the diet) and to change \( \Delta P \) in diet polyunsaturated fatty acid. It was assumed that diet cholesterol plays no role, that all saturated fatty acids have the same hypercholesteremic effects, that all polyunsaturated acids have the same hypocholesteremic effects, that the contribution of monoenoi acids is insignificant, and that all persons respond alike. This equation was further developed later in 1957 (31). The data from which the equation was derived included studies in which natural fats, either containing or free of cholesterol, were used at levels of 9 to 44% of the diet calories over periods of 2 to 4 weeks. These authors could not find confirmation of their formula in comparison of natural and hydrogenated safflower seed oil (83). Keys et al. (82) had conditioned their equation: "We conclude that the numerical values of equation 4 \[ \Delta \text{chol} = 2.74(\Delta S) - 1.31(\Delta P) \] apply only to persons whose serum cholesterol concentration is of the order of 225 mg/100 ml on an average American diet." They also admit that the equation is only valid for groups of men, but not for individuals, because of large individual variations in response to diet fat.

Two years later, these authors (85), again taking cognizance of "intrinsic differences among men," but still refusing to concede any contribution by diet cholesterol, collated data to test the equation for individual variations. From their own data and from selected literature values, they arrived at the equation \[ \Delta \% = 1.91x\% - 91 \], where \( x\% \) is the cholesterol value of an individual expressed as percentage of the average of a group of men on the same diet, and \( \Delta \% \) is that individual's cholesterol response in changing to another diet, also expressed as percentage of the group response to the same dietary change.

In short, the equation purported to show that "the average cholesterol responses of groups of men to changes in fat in the diet can be satisfactorily predicted from knowledge of the amounts of fat in the diet, and that such a prediction can be made even for groups of men who are relatively hypercholesteremic or hypocholesteremic."

Eight years later the same three authors, in a series of four papers (32), reviewed their equations from the standpoints of 1) the degree of unsaturation of the fatty acids, 2) the effect of diet cholesterol, 3) individual variations among persons, and 4) particular fatty acids.

Suffice it to say that they now realize that all these variables influence serum cholesterol and must be considered in their equation, but they do not yet take plant sterols into account. They concede that saturated acids of less than 12 carbon atoms or more than 16 are neutral, in distinction from the Harvard group (80) who have changed their minds about a hypercholesteremic effect of lauric acid, which they now exonerate.

Although the Harvard group have themselves devised regression equations, they admit that "Regression equations are simply descriptive. . ." (italics theirs). "The equations do not prove that fatty acids had the effects (80)."

Ahrens et al. (85) have also criticized the use of multiple regression equations as proof of the hyper- or hypocholesteremic proper-
ties of specific fatty acids. Ahrens pointed out that other equations could just as well fit the data, using oleic acid values instead of linoleic acid, for example. In that case, it could be made to appear that linoleic acid is neutral and that oleic acid is the hypcholesteremic constituent of the fats.

A third group prominent in this field has also found that the Keys formula does not fit their data (38). In their study of the interrelated effects of diet cholesterol and fat (described in some detail elsewhere in this critique), this group found in three experiments that, whereas the formula would predict no change, -32 mg/dl, and no change, respectively, in serum cholesterol, the changes found were -38 mg/dl, -2 mg/dl, and +28 mg/dl.

Other examples of failure of experimental data to fit the formulas are mentioned in the body of this report, e.g. (30).

A recent paper by Grande et al. is an example of how one can be misled by a questionable major premise (3). Presuming that the Keys formula is correct (\[\text{chol} = 1.2(2S-P)\]), they rationalized that all fats which contain twice as much saturated as polyunsaturated fat should produce the same serum cholesterol concentrations. Using various mixtures of vegetable oils, all with a P/S ratio of 2:1, they found no difference in serum cholesterol, regardless of different proportions of 12, 14, or 16 carbon fatty acids. This, is contradictory to the conclusions of other supporters of the saturated fat theory: that lauric acid has no effect, that myristic has little, and that only palmitic (under certain circumstances) is strongly effective (80, 81).

One can only interpret the data in this paper to mean that all fatty acids act alike and that none has any effect on serum cholesterol, although the authors claim to have demonstrated the validity of the Keys formula in which all saturated acids have the same weight.

The most ardent advocates of the saturated fat theory cannot agree. The Minnesota group conclude that stearic acid can neutralize palmitic in cocoa butter, although they still would equate \(C_{12}, C_{14}\), and \(C_{16}\) in other fats. The Harvard group believe that lauric acid, \(C_{12}\), is also neutral, and that myristic plays a minor role. They still support palmitic except that in some fats such as cocoa butter and olive oil, they grant that palmitic is neutral. Wherein, therefore, lies the hypercholesteremic property of saturated fat?

**Comments**

Perhaps the most telling argument against the theory of a hypercholesteremic response of serum cholesterol to diet saturated fat comes out of the effort to pinpoint the responsible fatty acids. It is agreed that acids of 12 carbon atoms or less are not involved, nor is stearic. Palmitic, it is also agreed, is not effective in such oils as olive and cocoa butter. How is it possible, therefore, to attribute the phenomenon of hypercholesteremia to saturated fatty acids?

It is interesting that although efforts have been made to elucidate the mechanism by which exogenous polyunsaturated fatty acids may lower serum cholesterol below the levels attained on a neutral diet, the reviewer is not aware of similar efforts to explain how exogenous saturated fat, as distinct from endogenous fat of the same fatty acid composition, has the opposite effect. If diet saturated fatty acids undergo a metabolic pathway different from endogenous (which could explain its purported effect on serum cholesterol), such should be amenable to experimentation.

Attribution of hypercholesteremia to a saturated fat, based on data obtained when it was contrasted to a polyunsaturated, phytosterol-containing vegetable oil, has been an error in experimental design leading to erroneous interpretation of results. Efforts have rarely been made to compare the two types of oils by relating each to a neutral diet. Preoccupied by the saturated fats, researchers have attributed differences to the saturated fats alone. It is relevant that in similar studies in which interest was on the unsaturated fats, all differences were often attributed to them alone.

The condemnation of "saturated fat" as the hypercholesteremic agent in animal fats
is a typical example of "guilt by association." The half-hearted efforts during the 1950's to disassociate the effects of the two constituents of animal fats resulted in exoneration of the cholesterol. Although it has gradually become acknowledged that cholesterol is the more responsible partner, saturated fat is still blamed in loose use of terms and remains the co-villain of the drama in the minds of the undiscerning.

The reported elevations in serum cholesterol concentrations accompanying saturated diet fats (when they contained little or no cholesterol) were rarely if ever great enough to produce hypercholesteremia in normocholesteremic subjects. That is, even when saturated diet fats did appear to result in somewhat higher serum cholesterol levels than the contrasting diets, the elevations were not to hypercholesteremic levels. The claimed responses to saturated fats were always well below those of the subjects on their ad libitum diets. Thus, saturated fats do not produce hypercholesteremia by any criterion, nor do they elevate it to high risk levels if indeed, they raise it at all.

Perhaps the key experimental oversights in these studies have been the failure to firmly establish the serum cholesterol concentrations on one diet before changing to another, and proper interpretation of the curves relating serum cholesterol to diet changes. Numerous cases were pointed out in which the changes had started before the institution of a new diet, and in which maximum, minimum, or average levels during a period instead of the final level, were considered as representative of the period. Associated with this is the lack of uniformity of the criterion of steady state and the lack of appreciation of the wide fluctuations that can normally occur. This has been pointed out by Wilkinson et al. (86) in their own studies: "Another reason for these discrepancies may be the wide fluctuations that occur in serum cholesterol over long periods of time which are unpredictable. In a typical case ... the mean of the control period was 272 mg percent against the mean during the "sitosterol" period of 258 mg percent. However, during the control period, of equal length to the period of 'sitosterol' administration, we could show a mean cholesterol value of 303 mg percent as well as 241 mg percent. We feel that these fluctuations present a hazard in the study of serum cholesterol-lowering agents. . . ."

Other oversights developed in the critique were the failure to consider the well-established effects of cholesterol and phytosterols, the differences between phytosterols (including their hydrogenated products), the lack of control over the actions of free subjects, inadequate consideration of caloric intake and weight changes, and inadequate numbers of subjects.

One of the reasons for serious errors in studies with humans lies in the inherent difficulty of establishing proper controls. One cannot obtain groups of standard people and divide them into subgroups. Although the measurement of the response to saturated fat is in the nature of a bioassay, none of the myriad of investigations, represented by those examined in this critique, meets the restrictions of a bioassay. Most of them disregard long accepted standards for the determination of nutritional values and of bioassays, not to mention the rigorous requirements for scientific proof. Few of the authors would accept conclusions of pharmacological action of drugs or even of food additives by such loose criteria.

One must be bold indeed to attempt to persuade large segments of the populations of the world to change their accustomed diets and to threaten important branches of agriculture and agribusiness with the results of such uncontrolled, primitive, trial and error type explorations. Certainly modern science is capable of better research when so much is at stake.

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