



Bias and misrepresentation revisited: Perspective^{1,2} on saturated fat^{1,2}

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Honest controversy can stimulate research and lead to more careful thinking; it is often a useful part of the process that builds the sure knowledge of science; many program committees and editors welcome controversy because it is one way of attracting an audience. But scientific argument must not be confused with the operations of opposing lawyers who select among facts, magnify or belittle them, and present what they label "evidence" in whatever light that seems best calculated to gain the day for the client. Scientists long ago eschewed that approach as the way to understanding. On the other hand, we can understand and accept something of the *ex parte* attitude that seeks to discover the weaknesses of a theory or set of conclusions with more enthusiasm than it tries to find the strong points. Differing persuasions can lead to different emphases but distortions are not permissible.

One of the definitions of "perspective" is "the aspect of an object of thought from a particular standpoint" (Webster's Third New International Dictionary, 1969). Things may look differently from different vantage points; the small may loom large, or vice versa. More consequential, vital features may be lost from sight entirely when the point of outlook is ill-chosen; in myopia, the trees conceal the forest. However, the disadvantages of a "particular standpoint" do not include the sight of things that do not exist, unless there is a perverse imagination. Besides, more important than the particularity of a single outlook is the idea that perspective brings a proper sense of proportion and balance; it continues to hold to the original meaning of the word, "looking through," so as to avoid distortion.

With any definition, it is impossible to accept

as a "perspective" Reiser's piece on "Saturated fat in the diet and serum cholesterol," (May 1973 issue of this journal). Nor can the second part of the title be taken at face value; it is certainly not a "critical examination of the literature," if "critical" is used in the common meaning in science as "careful," or "scrupulous" rather than the more literary use to mean "censorious." For from beginning to end, Reiser's article is censorious instead of critical; it is characterized by a complete lack of balance; it "looks through" a distorting system that reminds one of the distorting mirrors in the hall of jokes at the county fair. Even the casual reader, unfamiliar with the details of the literature Reiser condemns, will recognize serious bias, but actual examination of the papers quoted is necessary to appreciate its depth. For the "critical examination" is largely a tissue of repeated distortions and misrepresentations enlivened by more than a few inventions of the writer.

Setting the stage

Reiser starts by deploring "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 . . ." (1). We do not believe that such a "message" was ever expressed by any of the scientists whose studies on the relationship of the diet to the cholesterol in the blood are attacked by Reiser. We, at

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least, would deplore that message, just as we object to propaganda to the effect that protection from heart attacks can be had by switching to this or that product loaded with polyunsaturated fatty acids. We also object to the subsidized teaching that we are in danger of protein deficiency unless we eat "plenty" of meat. Furthermore, we deplore the message put out by all the media in every corner of the land that we are in danger of ill health unless we drink milk because, in letters a foot high, "EVERY BODY NEEDS MILK."

An outcry against some advertising excesses in food industry, based on identified fact—what? where? when?—could serve a useful purpose. But Reiser's target is not the food industry; his article is an attack on scientists who have nothing to do with industry; it is an attempt to discredit them, their experiments their observations, and conclusions. It is typical of the article that the scientists whose work and views he attempts to destroy are presmeared at the outset by the implication that they cry a dangerous and totally unwarranted "message" which foments "extremism and unbalanced diets" and produces "adverse economic consequences."

The same kind of attempt to injure the status of adversaries by transfer is indicated in the second page (p. 525) of Reiser's article where, "Before embarking on the critical evaluation of the supporting literature," he inveighs against "imprecisions" in terminology. The implication, of course, is that the authors whose works he is about to evaluate were guilty of the steps "away from accuracy and toward confusion," he lists on p. 525. We deny that such "imprecisions" actually characterize the relevant scientific literature. We would agree with Reiser when he objects to the loose characterization of a food fat simply as "saturated" without further specification but what, then, is to be said about his own statement a few lines later: "However, animal fats can be quite polyunsaturated if polyunsaturated fats are included in the animals' diets." What, please, is "quite polyunsaturated"? Is it enough just to have some polyunsaturated fats in the diet? What animals are meant? All animals? The cows and beef cattle that pose the great question about saturated fat in the American diet? It would be difficult to pack more imprecision in a 16-word sentence.

The next sentence in the same paragraph is at least equally unacceptable, not only because it is imprecise but because it conceals a personal conclusion demonstrably unwarranted: "Eggs, even when high in linoleic acid, maintain their hypercholesteremic property because of their constituent cholesterol." The proof of that "because" is indicated to be in the reference to a paper by Brown and Page (2). That paper reports on five "normal" young men who changed from their usual American type diet to a diet in which 75 of a total of 90 g fat in the daily diet were provided by unhydrogenated vegetable oil. Serum cholesterol decreased nicely in the 18 days of the trial. When the experiment was repeated with the addition of two egg yolks daily, there was no such consistent change in cholesterol. In a third trial, a repetition using eggs in which polyunsaturated fatty acids represented 45 instead of the usual 13% of the total glycerides in the yolks, the cholesterol response was not much better.

The data published by Brown and Page show that saturated fatty acids provided 12% of calories in the no-egg diet, 13% in both of the egg diets, whereas polyenes were lower in both of those egg diets than in the plain vegetable oil (control) diet. Instead of Reiser's unqualified "because," it must be noted that with no consideration at all of exogenous cholesterol the plain vegetable oil diet would be expected to have more cholesterol-lowering power than the egg diets because it contained less saturated and more polyunsaturated fatty acid. Furthermore, it would be expected that the serum cholesterol effect of the two egg diets would not differ because they were identical in fatty acid content.

About epidemiological studies that support "the saturated fat theory," Reiser says, "they will not be reviewed here," which does not deter him from stating that "there are probably more epidemiological reports to the contrary" (1). This is an easy way of trying to implant his own view without the need for citations that could be looked up to see what the evidence might actually be. In any case, it is gratuitous to write: "It is incorrect to quote epidemiological data as tests of the hypothesis." Who has been making such statements about "tests of the hypothesis?" The actual statements about the findings of epidemiological inquiries are, and should be, that they are (or are not)



"consistent with the hypothesis." For example, in a major publication we wrote: "Such findings do not prove that in the development of coronary heart disease saturated fats in the diet play a major role but at least they are consistent with that hypothesis" (3).

The setting of the stage for Reiser's more detailed discussions, or, better, distortions, was completed by his listing "some of the more common" faults in the various studies he is at such pains to discredit, stating that "these will be brought out with each paper reviewed." The implication is that many or all of the seven egregious mistakes or stupidities he listed characterizes the whole of the literature that supports the conclusions he hopes to destroy. It is grossly unfair, to use the gentlest label possible, to characterize the more serious studies in that way, the studies that did allow enough time and involved enough subjects, that did control body weight and food intake and adherence to the prescribed regimen. These remarks cover four of Reiser's list of seven. We shall deal later with a fifth point (Reiser's number six) concerning the alleged failure to consider "the effect of hydrogenation on the phytosterols in hydrogenated oils." It is enough to state here that this point cannot apply to the great majority of the experiments because hydrogenation was not involved in them.

Two of Reiser's points remain. Number two on his list is "attribution of differences between saturated and polyunsaturated to the former when the effect could be due to the latter." The possibility of such confusion arises in experiments in which saturated and polyunsaturated fatty acids are exchanged in the diet. We ourselves made, and reported, many controlled experiments of this kind. It is not true, as stated by Reiser, that we were swayed by "preconceived notions" about the culpability of saturated fatty acids. As early as the mid 1950's we considered the possibility that the fall in cholesterol in such exchanges could be due to a specific effect of linoleic acid in the diet. It was intriguing to speculate that high blood cholesterol levels could be reflections of a deficiency of essential fatty acids in the diet (4). That idea was destroyed by demonstrations by ourselves among others that fish oils (5, 6) and whale oil (7) containing polyunsaturates but no linoleic acid had an effect similar to that of corn oil when substituted in the diet for

more saturated fats. (Incidentally, in regard to reference (7), Reiser says (p. 529) that he had to estimate cholesterol "from a line chart in which the scale is 2 mm for 100 mg/dl." This is typical of Reiser's accuracy; the scale is closer to 20 mm for 100 mg/dl). Furthermore, it was clear that polyunsaturated fatty acids alone could not be responsible for serum cholesterol changes because the serum cholesterol level fell when the diet was made low in saturates and total fats, the polyunsaturates being not appreciably increased (8, 9). It is impossible to credit polyunsaturated fatty acids with the dramatic decreases in serum cholesterol produced by extremely low fat diets such as the rice-fruit diet (10, 11). Reiser would credit the absence of cholesterol for the effect with the rice-fruit diet but that ignores the fact that adding vegetable fat margarine to the rice-fruit diet evoked a prompt rise in serum cholesterol (11). It should be noted that the margarine of 1949 to 1950 was high in saturated fatty acids.

Illumination came when multivariate analyses were made, not as stated by Reiser, incorporating "pre-conceived notions." Data were at hand from many dietary experiments on men in locked buildings in mental hospitals in which the same subjects subsisted on experimental diets varied in a Latin-square design, diet cholesterol constant, no change in mode of life, and dietary calories stabilized to maintain energy balance with no change in body weight; the focus was on isocaloric exchange of fat for starch or of one kind of fat for another. The question was then asked: How can the observed changes in serum cholesterol be best accounted for from considerations of the proportion of total calories supplied by glycerides of saturated, monoene, and polyunsaturated fatty acids in the diets (8)? This question oversimplifies the matter of fatty acids, of course. No attention was paid to chain length and no distinction was made among various polyunsaturates; such details could be considered later if the multivariate approach seemed to be revealing. Least-squares solution of the multiple regression equation with those three major classes of fatty acids as the independent variables gave a mathematical answer. In short, monoene could be ignored, it was equivalent in cholesterol effect to equal calories of starch in the diet; saturates increased the cholesterol level, whereas polyunsaturates



worked in the opposite direction but per unit weight or molecule they were only one-half as effective as the saturated fatty acids. Repetition of such multivariate analyses with many more sets of data gave essentially the same result (12, 13). Later experiments confirmed the general rule for most ordinary diets but added the refinement that the effect of saturated fatty acids in natural human diets in raising serum cholesterol is mainly or wholly due to lauric, myristic, and palmitic acids (13, 14). With most natural diets this limitation makes little difference in the calculation of the serum cholesterol change expected from a dietary change because the saturated fatty acids with fewer than 12 carbon atoms in the chain and those with more than 16 make up no more than some 5% of the total fat.

Reiser's remaining category of "error" in the work he challenges is number one on his list: "failure to consider the effect of plant sterols and cholesterol." This is, in fact, the backbone of his counter-theory; it will be examined in detail later.

Reiser's final touch in setting the stage before he gets down to cases is his statement on p. 526: "Thus, the early workers learned what more recent investigators seem to have forgotten. . . the role of neutral fat is indirect and secondary to that of cholesterol. . ." This is a typical distortion; the "fact" is only in Reiser's mind. Of course, it has long been known that dietary cholesterol is poorly absorbed in the best of circumstances and is scarcely absorbed at all in the absence of a fat vehicle. But this is nothing like proof that the neutral fats have only a secondary influence through their effect of the absorption of exogenous cholesterol. And the contention disappears entirely in the face of the results of experiments with diets free of cholesterol in which changes in the dietary glycerides are followed by marked changes in the serum cholesterol level.

There is no lack of data on dietary fatty acid changes in experiments that did not involve any change in cholesterol in the diet yet were productive of changes in the cholesterol in the blood. Take an example from an experimental situation that even Reiser admits was "well-controlled" (p. 536). A cholesterol-free liquid formula diet, providing 40% of calories from fats, was fed to prisoners for three successive 3-week periods (15). In the first and third

periods the fat was cocoa butter, in the second period it was corn oil redistilled "so that the plant sterol content of the corn oil and cocoa butter were approximately equivalent." The group means and standard errors for serum cholesterol at the ends of the dietary periods were 222 ± 13 and 225 mg/dl for the cocoa butter periods, 177 ± 14 mg/dl for the corn oil period. Analysis of the findings by pairs of the means of the individual men on the two different kinds of fats gives: first cocoa butter minus corn oil, mean difference = 45 mg/dl, $t = 7.34$, $P = 0.0007$; corn oil minus second cocoa butter, mean difference = -48, $t = 14.17$, $P = 0.00003$. Reiser dismisses these ugly facts by noting that the two diets differed by some 111 mg of plant sterols daily, an amount, as will be shown shortly, only one-eighth that claimed to produce any effect in the most favorable report in the literature. In that report, a difference of 12.7 mg of cholesterol per 100 ml of serum was said to be produced by a difference of 870 mg of sitosterol in the daily diet.

Another study on prisoners, 30 "normal" men, involved substituting either safflower or coconut oil for 80% of the fat in the ordinary prison ration (16). Each fat was used for 1 month and there was 1 month on the uncontrolled prison ration in between, as well as 1 month before and after the experiment. The mean serum cholesterol value at the end of the safflower period was 165 mg/dl, at the end of the coconut oil period it was 203; the mean difference of 38 mg/dl has a standard error of 8.51, $t = 4.46$, $P = 0.0001$.

Reiser objects because the cholesterol values on the ordinary uncontrolled prison ration were variable, with averages of 201, 171, and 200 for the three sets of blood samples. No matter what efforts are made to manipulate the data, a major difference persists between cholesterol levels on the two kinds of oils. Compare the average for all three periods on the ordinary prison ration with the values for the same men on safflower oil; the result is $190.7 - 165 = 25.7$, with $t = 3.21$, $P = 0.003$. The corresponding comparison with coconut oil is $190.7 - 203 = -12.3$, $t = 1.47$, $P = 0.152$. In other words, replacing almost all of the meat fat and butter fat of the ordinary prison ration with coconut oil produced an increase in serum cholesterol that might be found by chance in approximately one out of seven repetitions of the



experiment. Changing from a diet high in saturates to one still higher, and with a concomitant decrease in exogenous cholesterol, would not be expected to cause a big increase in serum cholesterol. The critical point is that there is no way to escape the fact that the difference between cholesterol levels on the two oils could be explained by chance in only 1 in 10,000 trials.

Reiser dodges the facts and goes on to invent new ones. He says, "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" (p. 541). A difference of 1.5 lb in a month is negligible, of course, but in any case the "difference" is Reiser's personal contribution. The actual publication reported a gain of an average of 1 lb on the safflower oil diet but said nothing about any gain on the coconut oil. No doubt Reiser would complain, also, that the two oils were not matched in phytosterols. Most likely there was a difference of several hundred milligrams of phytosterol a day. It is time to examine the facts about the influence of plant sterols in the diet on the concentration of cholesterol in the blood serum.

Plant sterols

Edible vegetable oils contain phytosterols at concentrations of close to 0.1% to as high as approximately 1.5% in wheat germ oil. In most vegetable oils, sitosterol is by far the dominant phytosterol and may represent as much as three-fourths of all the nonsaponifiable matter. The phytosterols are interesting because they are chemical relatives of cholesterol and, taken by mouth, they are nontoxic and are only absorbed in the gastrointestinal tract to an extremely limited extent.

In the early and mid 1950's, commercial interests spurred much research on the possibility of control of serum cholesterol by orally administered plant sterols, particularly beta sitosterol. A summary of 18 studies on man reported up to 1957 showed that the dosages ranged from 5 to 45 g phytosterols daily (17). Sixteen of those reports indicated at least some average serum cholesterol reduction, whereas two found no effect. A later careful study, based on 18 g of beta sitosterol as most likely

to succeed, found with that dose there was a modest but statistically significant effect (18). Since then, interest in this approach to cholesterol reduction has evaporated because such large amounts of sitosterols are needed to affect serum cholesterol in man. As of 1963, phytosterols were not considered of potential use in lowering cholesterol, as only one agent, nicotinic acid, is credited with having an effect (19). A more recent review states: "Unfortunately, very large doses must be used and prolonged treatment is expensive. According to most reports the effects are variable" (20). A review in 1972 does not even mention plant sterols in the management of hyperlipoproteinemias (21).

The literature on the effect of phytosterol in man subsisting on natural foods is in agreement that the lowest amount that can be hoped to produce a discernible reduction in serum cholesterol is approximately 6 to 10 g daily. With a formula diet in a trial of only 8 days, the lowest effective dose was reported to be 870 mg daily (22). However, in that experiment, daily doses of 5,500 and 8,000 mg produced cholesterol declines of only 25 and 29 mg/dl. The brevity of the trial and the absence of natural foodstuffs in it makes it impossible to be guided by that report as opposed to 19 other studies that 10 or more times that dosage is needed. Note that 870 mg of phytosterols correspond roughly to the amount in 100 g of corn oil, the richest source of phytosterols among common food fats.

It is interesting that Reiser emphasizes an important role of the plant sterols in food oils in dozens of places in his article but says nothing about the foregoing, that is to say, the amount needed to affect serum cholesterol. Nor does he discuss the concentration of plant sterols in food oils except to state that "corn oil is composed of nearly 2% sterols." A review of the chemical literature indicates values for corn oil ranging from 580 to 1,000 mg/100 g, an upper level, in other words, of nearly 1% (23). In our laboratory, we have obtained values of 0.8% to 1.5% for total nonsaponifiable matter in corn oil. Other common food oils such as cotton seed, peanut, safflower, sesame, and sunflower seed average perhaps one-half the concentration in corn oil, whereas olive, palm and coconut oils are lower still.

Repeatedly, Reiser expresses his view that the cholesterol depression observed when corn

oil replaces a more saturated fat in the diet is the result of the phytosterols in the corn oil. Besides ignoring the mountain of literature noted above on the quantity of phytosterol needed to produce a drop in cholesterol, Reiser paid no attention to the one attempt to evaluate the effect of the unsaponifiable matter (largely sitosterol) in corn oil (24). The experiments involved 24 male schizophrenic patients in a crossover design of diet change with the diet constant except for isocaloric substitution for carbohydrate of 55 g of oil daily. The oil was a mixture of cottonseed and safflower oils with a fatty acid composition similar to that of corn oil. This oil was fed as such or with the addition of 880 mg daily of the unsaponifiable matter of corn oil, i.e., a rough estimate of 800 mg phytosterols. The mean values for the 26 men were 207 mg/dl, SD = 36.3 on the control diet; 166, SD = 25.6 on the plain oil; 163, SD = 23.2 on the oil plus unsaponifiable matter. The difference of 3 mg/dl that might be attributed to the phytosterol of the corn oil is associated with SE = 3.1; the trivial difference does not approach significance.

Hydrogenation

Reiser is aware of the great threat to his contentions from experiments showing a response of serum cholesterol to hydrogenation of the fat but with no change of cholesterol in

the diet. His answer is that phytosterol can reduce the serum cholesterol and "the possibility that hydrogenation may destroy this effect appears not to have been considered" (p. 526). Suppose we partially hydrogenate a fat such as sunflower seed oil and compare its effect in the diet with the unhydrogenated oil. The comparison, then, is between two fats that differ by no more than approximately 300 mg unhydrogenated phytosterol per 100 g of fat. Suppose those fats are fed to supply one-fourth of total calories, or 90 g/day in a rigidly standardized prison diet. What effect on serum cholesterol would be expected to result from the difference in phytosterol? As noted above, there is plenty of guidance. From all but one of the 20 reports in the literature, the answer is that 300 mg of phytosterol a day is perhaps one-twentieth the amount needed to produce a barely discernible lowering (17, 18). From the other, from the most optimistic report (that of Beveridge et al. (22)), 300 mg phytosterol/day is barely one-third the amount needed to show any effect.

Above we have, in effect, described the experiment of Antonis and Bersohn (9). Table 1 summarizes their findings. There were four experiments; Reiser managed to compress these into two by averaging and without informing the reader, and he quoted standard deviations rather than standard errors which has the effect of suggesting less than the actual significance of

TABLE 1
Serum cholesterol concentration in prisoners in four pairs of experiments

Duration, weeks	Race	Fiber, g/day	Fat	No. of men	Serum cholesterol, mg/dl					
					Mean	SD	Difference		<i>t</i>	<i>P</i>
							Hy-oil	SE		
22	White	11.6	Hy	11	212	45				
22	White	11.6	Oil	9	162	22				
22	White						50	16.45	3.03	0.014
29	White	3.6	Hy	10	207	35				
29	White	3.6	Oil	10	157	24				
29	White	3.6					50	13.42	3.72	0.004
22	Bantu	14.2	Hy	8	186	24				
22	Bantu	14.2	Oil	9	144	26				
22	Bantu	14.2					42	12.19	3.44	0.009
29	Bantu	5.0	Hy	10	191	23				
29	Bantu	5.0	Oil	10	149	26				
29	Bantu	5.0					42	10.98	3.82	0.003

In each experiment, the only dietary difference was in the character of the experimental fat supplying 25% of the total calories, or an average of approximately 90 g/day. "Hy" is hydrogenated sunflower seed oil, "Oil" is plain sunflower seed oil. Data from Antonis and Bersohn, *Am. J. Clin. Nutr.* 10: 484, 1962.

the serum cholesterol change. Then he makes the extraordinary statement: "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."

Three comments must be made. First, there is not the slightest justification for Reiser's proposal that the prisoners, any of them, found access to other foods. That idea would be just as applicable to the prisoners studied by McOsker and colleagues (25), whose results please Reiser more and therefore elicit his praise, "one may have confidence in the recorded dietary regimens" (p. 532). Second, talk about "the customary high cholesterol food of the Bantu," reflects gross ignorance or deliberate deception. The "customary" food of the Bantu, in or out of prison, is extremely low both in cholesterol and in saturated fatty acids. It was knowledge of that fact that impelled us to study them in South Africa (26). That dietary peculiarity of the Bantu has been found in every survey, in every examination of their kitchens and calculation of the use of the food they grow and buy; it is, in fact, ample explanation of the remarkably low serum cholesterol values that characterize them. Finally, Table 1 shows how Reiser distorted the facts by writing that the finding indicated only "the 'suspicion' of significance." Each of the four sets of comparisons shows a highly significant difference. The odds indicated from considering all four sets of independent experiments are astronomical, of course.

Reiser concluded his attempted discreditation of the 2-year study of Antonis and Bersohn (9) by stating, "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" (p. 532). Consider the last point first. How is it possible to say that "loss of linoleic acid can explain," but deny that the saturated fatty acids could be involved? Here again is a situation in which both saturated and polyunsaturated fatty acids were changing in opposite directions. Without other information it is impossible to blame one rather than the other. From the results of our own multivariate analyses the prediction is that serum cholesterol

would rise because of both changes in the dietary fat. As for the argument about the hydrogenation of the plant sterols, the facts brought out in the preceding section of our discussion should have made it clear that a difference of perhaps 300 mg phytosterols could not possibly make any difference. Besides, hydrogenation does not remove or destroy phytosterols, and there is not a shred of evidence that hydrogenation of phytosterols affects their influence on the blood cholesterol. Because Reiser repeatedly makes such a point about this purely imagined idea, it is high time that he produces some evidence, some data, showing what, if anything, is the difference between hydrogenated and ordinary sitosterol in effect on blood cholesterol.

More than four full columns of Reiser's article are devoted to the attempt to deny two of the pioneer reports that higher cholesterol values in the serum result when man is fed hydrogenated vegetable oils than when the natural oil is used. Bronte-Stewart and colleagues (27) made the first report. Only a single Bantu man was the subject in a metabolic unit so it may be asked whether the findings are generalizable to other men, but first it is necessary to ask what happened to that one man. We shall see that Reiser completely misrepresented the facts. Because both the senior and the second author of the paper from Cape Town are dead, we feel obligated to point out the truth of the matter. Reiser wrote (p. 527): "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 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. . . ." The facts are there for anyone to see in Fig. 2 of the original paper and in the text. First, nowhere in the paper is there a word to suggest that the authors took the value of 160 mg/dl "as representative of the response" to the hydrogenated oil; this is another of the many inventions by Reiser. Second, it simply is not true that "by the 12th day on the same diet the value had fallen back to 130 mg/dl." The last two values in the first experiment with the hydrogenated oil were identical, both were 148 mg/dl. After changing to the natural oil diet, the first blood sample gave 132 mg/dl and the values for the succeeding samples were 123, 120, 123, and 123 mg/dl in that order. In the second period on the hydrogenated oil diet, the cholesterol values were, successively, 140, 146, 149, 144 mg/dl. Reiser must have trusted that his readers would not bother to examine the original paper so as to learn the truth.

In any case, Reiser says that: "Without controls, the best interpretation of these data is that the serum cholesterol values represent normal variations. . . ." That idea is easily tested with standard statistical methods. Including all of the cholesterol values for each of the diet periods we have: 1st hydrogenated period, $n = 8$, mean = 146.5, SD = 9.01; natural oil period, $n = 5$, mean = 124.2, SD = 4.55; 2nd hydrogenated period, $n = 4$, mean = 144.8, SD = 3.78. Here is the analysis of the differences: 1st hydrogenated minus natural oil, mean = 22.3, $t = 5.09$, 11 degrees of freedom, $P = 0.0004$; natural oil minus 2nd hydrogenated oil, mean = -20.6, $t = 7.23$, 7 df, $P = 0.0002$. Actually, these are underestimates of the difference in effect of the two fats because in each period the first cholesterol value is from a blood sample drawn only 1 to 2 days after changing the diet, much too soon to approach the full effect of the dietary change. Omitting, then, the first blood sample in each period, the means prove to be 148.6, 122.3, 146.3 for the 1st, natural, and 2nd hydrogenated oil periods. The analysis, as above, shows mean differences of 26.3, $t = 6.89$, $P = 0.00007$ and -24.0, $t = 16.00$, $P = 0.00002$.

Reiser did not mention that between the natural oil and the 2nd hydrogenated oil periods there was a period in which olive oil was the fat in the formula. The successive cholesterol values were 120, 112, 111. Statisti-

cal analysis shows that the mean decrease of 9.9 mg/dl from the natural peanut oil diet is significant with P less than 0.03. This interesting result is roughly what we, but not Reiser, would expect because olive oil contains only approximately one-half as much saturated fatty acid as peanut oil. Incidentally, olive oil is lower in phytosterols than peanut oil so Reiser's prediction should have been a rise in serum cholesterol when the diet was changed from peanut to olive oil.

Reiser also failed to let his readers know that there were other experiments comparing the cholesterol effect of hydrogenated and natural oil in the metabolic unit of the Department of Medicine of the University of Cape Town. In regard to hydrogenated versus natural peanut oil, the *Lancet* paper (5) states, "The same effects were seen later in the same person and in 2 others." However, Reiser does state, "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." Not a word allowing such "information" is given in our copy of *Lancet* of April 21, 1956. That Bantu may have been "uneducated" (education was not mentioned in the *Lancet* article), but in any case it is an affront to sociological decency to propose that honesty is proportional to education. The statement that the subject "was not isolated but continued with his normal routine" is another of Reiser's inventions.

The experiment of McOsker et al. McOsker et al. (25) fed prisoners on four formula diets containing different proportions of partially hydrogenated fats and a formula with no hydrogenated fat. Reiser expressed satisfaction with that study, concluding: "Thus, hydrogenation did not produce saturated acids with hypercholesteremic activity, nor do the polyunsaturated fatty acids have hypocholesteremic attributes" (p. 533). It would be useful to have comments from Drs. McOsker, Mattson, or Klingman, but Reiser's major misunderstanding should not be allowed to mislead the trusting reader.

The experiment of McOsker et al. provided valuable information on the question to which it was addressed, namely the relative effect on serum cholesterol of *trans* fatty acids as compared with their *cis* isomers. The fats tested



were selected to provide contrasts on that score; the experiment was not designed to answer the question as to whether a hydrogenated fat in the diet would have an effect on cholesterol different from that of the same amount of the unhydrogenated fat in the diet.

McOsker et al. state: "A difference of 12 mg/100 ml serum represents a significant difference at the 95% confidence level ($P = 0.05$)" in their experiment. With that criterion, they found no significant difference between the cholesterol levels on any of the five vegetable fat diets. In those diets, there was no cholesterol and fat represented 41% of the total calories in each diet so it is easy to calculate the expected cholesterol differences from our equation (12, 13): $\Delta \text{Chol} = 1.3 (2 \Delta S - \Delta P)$, where Chol is in milligrams/deciliter and S and P represent the percentages of the total diet calories furnished by saturated and polyunsaturated fatty acids, respectively.

The greatest contrast in saturated fatty acid concentration in the diets of McOsker et al. was between diet A 114 with 25%, and diet B 109, with 14% of the fat being saturated fatty acids. The fats in those diets also differed in polyunsaturates, 56% versus 38% of the total fat, respectively. As fats provided 41% of total calories in all of the diets, the calculation of the cholesterol difference on diet A 114 versus B 109 is:

$$\text{Chol} = 1.3[2(0.41)(25 - 14) - 0.41(56 - 38)] = 2.1 \text{ mg/dl}$$

The observed difference was 0.3 mg/dl. Neither the observed nor the expected difference is significantly different from zero and, of course, they do not differ from each other.

Table 2 summarizes relevant data and calculations for the five diets. Note that instead of tabulating all 10 sets of differences between pairs of diets, the material is compressed by using the mean of the five diets as a common reference. In no case is there indicated a significant difference between observed or predicted cholesterol values on the several diets. Reiser's satisfaction with the findings of McOsker et al. is based on his failure to comprehend the material.

Experiments in Minnesota. In the discussion of the experiments with feeding ordinary oils versus partially hydrogenated oils in a metabolic unit in Minnesota (28), Reiser reveals his

TABLE 2

Mean serum cholesterol, milligrams/deciliter observed and that predicted from $\Delta \text{Chol} = 1.3 (2\Delta S - \Delta P)^a$

Diet	Percent of fat		Percent calories		Serum cholesterol	
	S	P	S	P	Observed	Predicted
A114	25	56	10.3	23.0	158.5 ±12 ^b	152.3 ^c
B109	14	38	5.7	15.6	158.2 ±12	150.0
C100	22	33	9.0	13.5	163.0 ±12	161.3
D95	24	29	9.8	11.9	158.4 ±12	168.0
E76	26	13	10.7	5.3	167.1 ±12	176.3
Mean of 5 diets	22.2	33.8	9.12	13.9	161.0	

^a S and P are percent of total diet calories from saturated and polyunsaturated fatty acids, respectively. Data from McOsker et al. (25). ^b Defines 95% confidence limits. ^c Compare diet A114 with five-diet mean: $\Delta \text{Chol} = 1.3 [2(10.3 - 9.1) - (23.0 - 13.9)] = -8.7$, hence predicted for A114 is $161.0 - 8.7 = 152.3$. This same method was used for the other predictions.

"grasp" of statistical theory. About experiment "K" he says: "Considering the normal variations for which datum is unavailable, these values are not significantly different" (p. 532). And again, in regard to experiment "N": "But even that [significance] cannot be judged as there was no control for normal variation." The same kind of remark occurs over and over again throughout Reiser's article. In the first place it simply is not true that information is "unavailable" about "normal variations" in experiment "K" or that there was "no control for normal variation" in experiment "N." Variation, including normal variation, is measured as dispersion, most commonly and appropriately as the standard deviation. Let us examine this matter a little.

Of course, there is "normal variation" in the concentration of cholesterol in the blood serum, as in all biochemical measures. We have repeatedly examined such variation in technical detail, intra- and interindividual variation, seasonal and other time trends, and the normal variations of laboratory error (8, 29). It is, or

should be, the business of the investigator to design experiments and control the analytical laboratory so that normal variations are not productive of systematic bias but are random and therefore subject to the theory of error. Normal variations dilute true differences and therefore make it more difficult to discern a true difference. With proper statistical analysis, normal variations do not create the illusion of a difference when there is in fact no difference. In comparing the outcome of two conditions, e.g., two diets, the problem of the experimenter is to prevent extraneous variables from exerting differential influences in those two conditions. Standard procedures to assure that normal variations are unbiased and therefore subject to treatment as random error include the use of the same subject in both conditions, arranging that one group of subjects change from condition A to condition B at the same time that a group of their counterparts makes the change from B to A, taking repeated samples and making repeated blind analyses in the analytical laboratory, and so on. The final result typically is, or should be, a mathematical statement that the difference between the values in conditions A and B amounts to a number which is associated with a numerical standard error at so many degrees of freedom. Then probability theory allows the calculation that the observed difference could occur by chance in a number of repetitions of the experiment. "Normal variations" have been evaluated and allowed for in the final probability statement.

So it is impossible to understand what Reiser means by his blithe remarks about "normal variation." He seems to have some private and personal definitions of "normal," of "variation," and of "significance" which have nothing to do with statistics and probability theory. Consider the result of experiment K in which 27 men showed a serum cholesterol average of 10 mg/dl higher on the partially hydrogenated than on the ordinary safflower oil. The standard error of that difference was 2.4 mg/dl to $t = 4.17$, and a difference of that magnitude could be expected to occur by chance with $P = 0.0003$. Conventionally, a value as small as $P = 0.05$ is considered "significant," a value of $P = 0.01$ is "highly significant," and so on.

The cholesterol difference in experiment K is not large, only 10 mg/dl, but then the change in

saturated fatty acids in the diet was small. The exchange fat only amounted to 30 g/day and the composition of that fat was 12% saturated fatty acids in the plain oil and 32% in the hydrogenated oil, the corresponding figures for polyene being 75% and 13%. In the period when hydrogenated oil was used, saturated and polyunsaturated fatty acids accounted for 21.5% and 3.2% of total calories, whereas in the natural oil period the figures were 19.8% and 8.6%, respectively. What is the expectation from our prediction equation?

$$\Delta \text{Chol} = 1.3[2(21.5 - 19.8) - (3.2 - 8.6)] = 11.4 \text{ mg/dl}$$

This is to be compared with the observed Δ Chol of 10 mg/dl.

In our experiment series N, the result of change of six men from natural to partly hydrogenated safflower oil and of six men changing in the reverse order was an average cholesterol rise of 25 mg/dl, the standard error of the difference being 4.4 mg/dl so $t = 5.68$. With 11 degrees of freedom, $P = 0.0001$. The larger effect of the exchange of hydrogenated for natural oil in experiment N than in K reflects the fact that the exchange fat amounts to 100 g/day in N instead of 30 g in K. In regard to experiment N Reiser says (p. 532) "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 . . ." That constituent cholesterol was the same on the natural and the hydrogenated oils and only amounted to nearly 100 mg/day according to Reiser's own estimate.

Reiser ignored the comparison between natural and partially hydrogenated corn oil in our experiment N. Fourteen men subsisted under fully controlled conditions for two successive periods of 21 days, seven men being first on natural corn oil and then on the hydrogenated oil while the other seven men subsisted on the two fats in the reverse order. Aside from the exchange fats, 100 g/day, the diet was constant over the entire 6 weeks. The percentages of total calories from saturated fatty acids were 9.6 and 12.6 on natural and on hydrogenated corn oil diets, respectively, the corresponding values for polyunsaturated fatty acids being 16.9 and 2.2. On the diet with the hydrogenated oil, the mean cholesterol level was 21

mg/dl higher than on the plain corn oil, SE of the difference being 3.8, $t = 5.52$, $P = 0.00008!$ Again, the expected serum cholesterol change can be calculated:

$$\Delta\text{Chol} = 1.3 [2(12.6 - 9.6) - (2.2 - 16.9)] = 26.9$$

the observed value of ΔChol was 21 mg/dl. In our original publication (28) we speculated that perhaps the cholesterol response to hydrogenation, slightly smaller than predicted, could reflect a little smaller effect of *cis* than of *trans* isomers. Actually, the difference between observed and predicted changes in cholesterol is not statistically significant.

Other experiments. The classic experiments at the Rockefeller Institute with patients on formula diets (30) are attacked at great length by Reiser. We defer to Ahrens and his colleagues to respond but we cannot refrain from commenting on Reiser's second paragraph in the 1st column on p. 528. Perhaps it is enough to quote the paragraph: "The authors...consider the differences significant. Obviously, they used averages, but as the values were steadily changing (though within the limits of $SD \pm 20$ mg/dl), one must consider that the final concentrations, not the averages, more truly represent the physiological responses." This is one of the more extraordinary of the many fantastic pronouncements in Reiser's piece.

Similarly, we defer to the authors of other papers attacked by Reiser to respond about hydrogenation: Drs. Malmros and Wigand at the University of Lund (7), Drs. Gordon, Lewis et al. at the University of Cape Town (31), Dr. Horlick at the University of Saskatchewan (32), Dr. Beveridge and colleagues at Kingston, Ontario (33, 34). However, we cannot resist some comment.

Writing about one of the experiments of Beveridge and colleagues, Reiser states (p. 530): "This is one of the rare studies in which the saturated and polyunsaturated fats are compared with a neutral diet rather than to each other, so that each can be assessed independently." Besides wondering what a "neutral diet" is we find it difficult to understand what is meant by "each can be assessed independently." Elsewhere, Reiser alludes in his article to some mysterious virtue of comparing a with b by a roundabout route of a versus c and b versus c. In connection with hydrogenation

what most people have wanted to know to begin with is the answer to the simple question: On a diet constant in other respects and with other aspects of the mode of life held constant, what, if any, is the difference in serum cholesterol level when a hydrogenated fat is exchanged for the same amount of the natural oil? Most of us would insist that the proper way to go about answering that question is to compare the cholesterol values of the same subjects on the two contrasting fats, preferably using two groups of subjects who make the diet change in reverse or crossover order so as to compensate for any possible seasonal physiological or analytical trend. We offer this advice to Reiser if he should ever get to the point of making an experiment on man.

Reiser also says, in regard to the experiment concerned in the preceding paragraph, "It is unfortunate that a sitosterol-free oil, such as sesame or a stripped oil, was not used as a control." We have already noted that Reiser's obsession on this point is without merit. The magnitude of possible sitosterol differences in such experiments is much smaller than that required to produce any effect according to the unanimous testimony of at least 20 experimental reports. We are interested to learn that sesame oil is "sitosterol-free." Our information is that in sitosterol concentration sesame seed oil is higher than peanut, cottonseed, coconut, soybean, and olive oils and is comparable to sunflower seed oil in this respect.

Cocoa butter and the stearic acid story

Reiser's lengthy discussion of cocoa butter (p. 534–538) follows the pattern of his treatment of other aspects of the diet fat-serum cholesterol question. We shall see how a grossly distorted picture results from selection and omission of facts in the original reports and the refusal or inability to consider statistical analysis.

Reiser says (p. 534): "Cocoa butter is a much more fair test of the saturated fat theory because its fatty acids are the same as those in animal fats and hydrogenated vegetable oils." This is not so. Cocoa butter does not contain the *trans* acids characteristic of hydrogenated oils nor the short-chain and branched fatty acids characteristic of milk fats. More important, it has a much higher content of stearic acid than other common fats, considerably



higher even than the indigestible tallows. Cocoa butter is not thought of as a food fat, though it is readily digested, and except for isolated trials, it was not used in systematic experiments on the effects of dietary fat on blood cholesterol in the period when most workers were interested in the polyunsaturates as a possible way of cholesterol control.

Ahrens et al. (30) found that substitution of cocoa butter for corn oil in a formula diet caused a rise in serum cholesterol but less than when butter was the dietary fat. The authors offered no explanation of these facts, seemingly at variance with their emphasis on iodine value as dominating the cholesterol response. Malmros (35) used cocoa butter to replace the meat and dairy fats in an ordinary Swedish diet. Cocoa butter is extremely low in linoleic acid so Malmros, then intrigued with the cholesterol-depressing effect of adding linoleic-rich fats to the diet, was surprised: "Despite this low linoleic acid content, we noted a certain decrease in the serum cholesterol in both cases" (35).

In the Netherlands, in a study in which corn oil and cocoa butter were compared as adjuncts to a diet primarily of lean meat, fish, potatoes and bread, changing to the corn oil diet from an ordinary Dutch diet produced a rapid and sustained fall in serum cholesterol (36). Change from corn oil to cocoa butter produced a cholesterol increase. Reiser says, "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" (p. 535). In the absence of consideration of the quantities of the various fatty acids in the diets, that reasoning is possible. The alternative, equally "reasonable," is to say, "all the changes may be attributed to the constituents of the cocoa butter, in which case one can conclude that corn oil is neutral." Even with this simplistic approach it is essential to define precisely what is meant by "neutral."

In the early 1960's several groups of investigators decided to use cocoa butter to represent a highly saturated fat in systematic dietary experiments. In our experiment AF our formula, $\Delta\text{Chol} = 1.35 (2 \Delta S - \Delta P)$, would have predicted a difference of 65 mg/dl but the observed difference was only 33, far too big a discrepancy for a fully controlled experiment

on 22 men subsisting on each of two diets in a switch-back design. Serum cholesterol did not increase as much as expected on the cocoa butter diet (37). In a second series of experiments, AM, the cocoa butter diet also failed to produce as much cholesterol increase as predicted. A similar series, experiment FM, was carried out on subjects of a different type in a metabolic unit at another hospital. Again, cocoa butter failed to produce as much cholesterol increase as expected. While we pondered these results of work lasting nearly 2 years, Connor et al. (15) published their data from similar experiments involving cocoa butter; their findings were in full agreement with what we had found. Almost at the same time the paper by Erickson et al. (38) appeared and again, using cocoa butter in the diet, there was a major discrepancy from what we should have predicted from our equation.

Reiser would propose that the equation is wrong. It could be simply that only a more exact definition of the variables is required. Data from 46 sets of experiments were available, using 4 from Connor et al. (15) and 7 from Erickson et al. (38). All this combined material was analyzed in terms of several mathematical models (37). The consensus is that the discrepancies between observed and predicted serum cholesterol changes disappeared when "saturated fatty acid" was defined to exclude stearic acid, the longest chain saturate commonly encountered in human foods. With that definition of "S," we were able to predict the cholesterol changes within the 95% confidence limits of the observed changes in the experiments of Connor et al. and of Erickson et al.

There still remained the possibility that cocoa butter contains some ingredient in small quantity that has a powerful cholesterol-depressant effect such that a cholesterol-promoting effect of stearic acid is counterbalanced. To test that idea, comparison was made between cocoa butter in the diet and an imitation cocoa butter made by mixing appropriate proportions of palm oil, olive oil, some totally hydrogenated soybean oil, and a little safflower oil. A third test fat was palm oil and a fourth was a mixture to match the palm oil except in having much of the palmitic acid replaced by stearic acid. These last two fats were randomized and deodorized at the Miami Valley Research



Laboratory of the Procter & Gamble Company. Reiser's Table 1 purports to summarize these fats but there are gross errors. What he calls "tripalmitin" was glycerides of saturated fatty acids with 12 to 16 carbon atoms. And what he calls "tristearin" was glycerides of saturated fatty acids with more than 16 carbon atoms. Total trisaturated triglycerides (in all 18-, 16-, 14-, and 12-carbon compounds) amounted to 3% in the cocoa butter but as much as 19% in the imitation cocoa butter.

The subjects, 30 mentally retarded men, were divided into four groups (8, 8, 7, and 7 men) matched for age, relative body weight, and serum cholesterol concentration on the standard hospital diet. The four experimental diets, a basal diet plus 83 g of one of the four fat supplements, were fed simultaneously for periods of 18 days to each of these groups of men. The diets were changed in successive dietary periods in a symmetrical Latin-square design so that by the end of the experiment each man had eaten each of the diets and each group had eaten the diets in a different order. The actual food intake of each of the individual men was recorded and the averages for each of the four diets were substantially identical. Mean body weight varied only from 67.2 to 67.5 kg on the four diets.

With analysis of variance, it was found that any between-diet difference in mean serum cholesterol concentration as great as 9 mg/dl is significant at $P = 0.01$. The results showed that the exchange of the cocoa butter versus its imitation was associated with a change of only 4 mg/dl (SE = +7.6). So a mixture made up from non-cocoa butter materials to imitate cocoa butter in fatty acid composition had a cholesterol effect no different from cocoa butter itself. Accordingly, the peculiarity of the cholesterol effect of cocoa butter in the diet is accounted for by its fatty acid composition. Furthermore, comparisons of the serum cholesterol values on the diets with palm oil and with its counterpart with changed proportions of palmitic and stearic acids were in accord with the proposition that stearic acid has little or no effect on the concentration of cholesterol in the blood serum (14).

Reiser wrote: "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 to establish variation with time. In short, there was no

control." This is either a remarkable demonstration of ignorance on the part of the author of the true course of cholesterol response in man to dietary change in regard to the elements of statistical theory or a deliberate falsification. Reiser is not so ill-informed as to be unaware of the fact that practically all, or actually all, of the serum cholesterol response in man to a change in dietary fat is completed in 2 to 3 weeks. When it suited his argument, he had no hesitation in citing the cholesterol changes in 7 or 8 days of dietary change; witness his reference (p. 530) to the experiments of Beveridge et al. (33, 34). As to the trend over time (72 days) Reiser either fails to comprehend the way that a Latin-square design of dietary change with groups prevents interference with such time trends or, again, he deliberately falsified the analysis of the published data.

Recognition of the fact that stearic acid does not share the cholesterol-raising effect of lauric, myristic and palmitic acids cleared up older discrepancies. Earlier, we had carried out two controlled experiments on physically healthy, middle-aged men with 140 g total fat in a diet providing 3,000 kcal/day (39). The fats of the two diets were closely matched in regard to saturated, monoene, and polyunsaturated fatty acids but one diet contained approximately 40 g more lauric and myristic acid than the other, whereas the second diet had almost 40 g more palmitic and stearic acid. The result of 50 comparisons showed that the diet with more palmitic and stearic acids produced an average serum cholesterol level lower than the other diet by 8 mg/dl with SE = ± 2.4 ; $P = 0.001$. Similarly, it became possible to explain older puzzles in the data of Ahrens et al. (30), Malmros (35), Horlick (32), and Horlick and Craig (40). What had seemed to be peculiarities in the data of Connor et al. (15) and Erickson et al. (38) proved to be precisely what would be expected when instead of S = all saturated fatty acids we used S' = saturated fatty acids with 12 to 16 carbon atoms in the chain.

Exogenous cholesterol

A major fixation of Reiser is the view that differences in cholesterol in the diets explains such contrasts in cholesterol effect as cannot possibly be attributed to phytosterols. When the cholesterol contents of two diets are

identical, recourse is had to the idea that exogenous cholesterol is absorbed to different degrees in the presence of different fats. The only evidence offered for this last idea is his citation of the paper by Filer et al. (41). That paper concerns the absorption of fat, not cholesterol, and shows that the fat absorption in newborn infants is less efficient when palmitic acid is randomly distributed in the triglyceride molecule than when it is in the 2-position. Filer et al. are at pains to note that this peculiarity of the young infant does not hold for older children or adults. The interest of that study was in the authors' apparent explanation of the fact that the fat in cow's milk is absorbed by small infants less well than the fat in human milk. Obviously, the only "evidence" cited by Reiser has nothing whatever to do with his claim. So let us turn to experimental data on the effect of cholesterol in the diet on the cholesterol level in the blood.

Man is so much less sensitive to dietary cholesterol than the rabbit and many birds that for many years it was doubtful whether ingestion of anything less than heroic amounts of cholesterol would have any effect on the level in the blood. Heymann and Rack (42) in 1943, reported that the serum cholesterol concentration in infants and children is independent of the amount of cholesterol in the diet, and there was no counter report. Messinger et al. (43) added enormous amounts of cholesterol, both as such and in egg yolks, to the diet of elderly men and found only modest serum responses of questionable statistical significance. A daily feeding of 2 g cholesterol in candy to pregnant young women failed to evoke a significant rise in the blood (44). Experiments on two male physicians and three interns (45) were summarized: "The diet low in fat and in cholesterol led to a highly significant decrease in plasma cholesterol levels, whereas the addition of cholesterol in the form of egg yolk did not cause any change." Furthermore, "The results of this investigation indicate that, within the limits used here, dietary cholesterol has no effect on plasma cholesterol levels" (45).

Reiser ignored all of the four studies mentioned above as well as our own report (46), which set forth the findings leading us to conclude that dietary cholesterol has no important effect on the cholesterol in the serum. It is proper to summarize that paper here. In dietary surveys, both in the United States and in Italy,

we found no correlation between diet cholesterol and the concentration in the blood serum. Furthermore, among 286 Minnesota business and professional men whose dietary habits were checked annually for 4 years, we compared 33 men whose estimated cholesterol intakes were consistently in the lower third of the distribution for all 286 men with 35 men who were consistently in the upper third of that distribution (46). The estimated mean daily intake of cholesterol in the diet by the high-intake men was 1,010 mg, that of low-intake men was 401 mg. Differences in the consumption of eggs accounted for almost all of the difference. The mean serum cholesterol values of the low- and high-intake men were 248.9, SD = 41.4, and 256.2, SD = 42.9, respectively. (These Bloor method values for cholesterol should be reduced by 10% to be comparable to values with newer, more cholesterol-specific methods.) The difference, 7.3 mg/dl higher for the high-intake men, has a standard error of 10.2 so it is far from being significant.

More persuasive to experimentalists may be the findings in completely controlled experiments in hospital metabolic units. Table 3 summarizes the serum cholesterol values of 26 men who changed from the standardized hospital diet to a modified rice-fruit diet or from the rice-fruit diet to hospital diet (46). On the rice-fruit diet, 14 men received a supplement of egg yolk providing 500 to 600 mg

TABLE 3
Serum cholesterol, milligrams/deciliter after 4 weeks on various diets^a

No. of men	Diet	Cholesterol	No. of men	Diet	Cholesterol
6	H	189.0	7	H	184.2
	RF	159.6		RF+	148.5
	Δ	29.4		Δ	35.7
	SE	±9.6		SE	±13.2
6	RF	158.2	7	RF+	173.0
	H	198.4		H	222.8
	Δ	40.2		Δ	49.8
	SE	±6.3		SE	±8.7

^a Standardized regular hospital diet (H), modified rice-fruit diet (RF), modified RF plus egg yolk (RF+), in the sequence shown above. Data from Keys et al. (46). The cholesterol values are the original Bloor method figures corrected by multiplication with the factor 0.87.

cholesterol daily, whereas the other 12 men on the rice-fruit diet received a supplement of all vegetable oleomargarine equal in fat to that of the egg yolk supplement. The dietary periods were 4 weeks each. Changing from the regular hospital diet to the rice-fruit diet without cholesterol produced an average fall of 29.4 mg/dl, whereas the change to the rice-fruit diet plus egg yolk produced a drop of 35.7 mg/dl. For the men who made the change in reverse order, rice-fruit to regular hospital diet, the average cholesterol level rose 40.2 mg/dl when the plain rice-fruit diet had preceded the regular hospital diet and 49.8 mg/dl when the rice-fruit diet had included the egg yolk supplement. In other words, the fact that the rice-fruit diet is cholesterol-free does not explain its cholesterol depressing effect.

In another controlled experiment on five men in a metabolic unit, after 4 weeks on a modified rice-fruit diet plus 500 to 600 mg cholesterol in egg yolk, the mean serum cholesterol concentration was 165 mg/dl. The men then continued on the rice-fruit without the egg yolk supplement for another 4 weeks; at the end of that time the mean was 166 mg/dl (46).

In another metabolic unit study, a basic relatively low fat diet (20% of total calories) included daily cookies free of cholesterol or providing almost 1,000 mg cholesterol daily. From other sources including a ration of butterfat averaging 34.4 g/day, the diet provided from 370 to 480 mg of cholesterol daily. Thirteen men had a serum cholesterol average of 184 after 4 weeks on the diet with the zero-cholesterol cookies, 186 mg/dl 4 weeks after the cholesterol-rich cookies had been used in the diet. Another 14 men had the cholesterol-rich cookies 4 weeks and then the zero-cholesterol cookies for another 4 weeks. The corresponding serum cholesterol averages were 187 and 179 (46). These findings suggest a slight effect, an increase of perhaps 3%, of adding 1,000 mg cholesterol to a daily diet that otherwise provided an average of 425 mg cholesterol/day.

Reiser ignored all the above cited studies that indicated only small effects of exogenous cholesterol on the blood and, in fact, cited only one negative report, that of Kinsell et al. (47). We agree that it is easy to criticize that study of one patient fed a synthetic fat; an experiment

on a single patient must always be looked at with reserve. As usual, Reiser's comments are misleading. In the first place it is ridiculous to state, as he did, that Kinsell's report about that experiment, published only in a 1956 letter to the editor of the *American Journal of Clinical Nutrition*, "was most influential in establishing the concept that diet cholesterol is not a factor in serum cholesterol levels but saturated fat is, and coconut oil is representative of saturated fat" (p. 539).

About Kinsell's patient, Reiser wrote: "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" (p. 539). We read the pre-experiment range as nearly 190 to almost 300; perhaps Reiser wished to minimize the subsequent fall. What "unconvincing" means is unclear; what is clear is that, with the exception of the first sample after starting the experiment, all of the nearly 20 successive samples showed cholesterol values below the minimum recorded before the diet change. It is also clear that the addition of 10 g cholesterol to this otherwise cholesterol-free diet produced only a trivial and statistically insignificant rise in the serum. Kinsell's experiment offers a poor basis for generalization but there is no ground for Reiser's statement: "Either the subject was a poor choice . . . or the serum cholesterol assays were in error, or both" (p. 539). The only explanation of that statement is Reiser's dislike of the results.

In 1960 Beveridge et al. (48) reported graded serum cholesterol responses in students on a synthetic formula diet for 8 days with daily cholesterol intakes of 13 to 3,441 mg. The brevity of the experiment, the use of the artificial formula food, and the fact the different amounts of cholesterol were fed to different individuals were obvious reasons for questioning whether the findings apply to persons changing to more normal diets of ordinary foods for longer periods of time. Then, from 1963 to 1964, three papers appeared showing that the serum cholesterol rises when change is made from a cholesterol-free diet to a diet otherwise comparable but containing 725 to 3,000 mg cholesterol in the daily ration (38, 49, 50). These three experiments were more satisfactory in that they were much longer and the subjects were their own

controls on two or more diets. All, however, depended entirely on liquid formula diets with no ordinary food items in them.

In 1965, we reported the results of experiments in a locked metabolic unit with 22 men in each of three experiments in a Latin-square design (51, 52). In each experiment the men, in four groups matched in age and serum cholesterol level on the standard control diet, subsisted on the experimental diet in four versions, differing in the amount of cholesterol. The basic diet, composed of vegetable products with the exception of 3 glasses of skim milk daily and 4 servings of fish weekly, contained only 17 to 19 mg cholesterol per 1,000 kcal. Supplements of 125, 181, 505, 507, and 540 mg per 1,000 kcal were tested. In each experiment, the order of diet change was in a switchback pattern, one group changing from diet A to diet B while another made the reverse change so that possible time trends unconnected with the diet would be compensated. In one experiment the duration on each diet was 2 weeks; in the other two experiments 3-week periods were used. These experiments differed from those reported in references 38, 48, 49, 50 in that natural foodstuffs were used, not liquid formulas.

The results of two of those experiments in Minnesota were analyzed, together with all the results reported in references 38, 48, 49, 50, for regression of the change in serum cholesterol, ΔChol , on change in diet cholesterol per 1,000 kcal (51, 52). The data of the third experiment were not used because the small response in the serum to large increases in dietary cholesterol might be attributed to the low fat basal diet, with only 8% of calories from total fats. Graphical display of the combined material from all five reports indicated a curvilinear relationship, with decreasing dose response at increasing dose levels, suggesting an exponential function with the exponent having a value less than one. Trial with the square root of the diet cholesterol change as the independent variable gave a reasonable fit to the data and the upshot was that least-squares analysis yielded ΔChol (milligrams/deciliter) = $-2 + 1.7 \Delta Z$, where Z is the square root of the cholesterol intake, milligrams per 1,000 kcal.

Reiser mentions none of this in his article, presumably because of the implication that dietary cholesterol is a good deal less important

than he would have us believe. Now one more study on the response of the serum level to cholesterol in the diet has been published (53) and this paper also was not mentioned by Reiser. Mattson et al. (53) fed four groups of 14 prisoners each on a high fat, liquid formula diet of egg white, dextrose, fat, salt, and water plus egg yolk or a fat supplement resembling the fatty acid composition of the egg yolk. In this way it was possible to compare the serum cholesterol response to the change from zero dietary cholesterol to 106, 212, or 317 mg cholesterol per 1,000 kcal, the diet being high in fats.

Mattson et al. (53) concluded that there was a linear increase of serum cholesterol with increasing dietary cholesterol. "Each 100 mg cholesterol in 1,000 kcal of diet resulted in approximately a 12 mg/100 ml increase in serum cholesterol." The data are shown in Fig. 1, which summarizes all of the relevant experiments in the literature, a total of 19 sets of values, including the three points from Mattson et al.

In Fig. 1, the data as a whole are reasonably concordant except for what seem to be two outliers, one from the study of Mattson et al. and one from the study of Connor et al. In the latter, it is notable that of two groups of young men making exactly the same dietary change, one group of six men exhibited an average rise of 38 mg/dl when the cholesterol was added to the diet, whereas the other five men had an

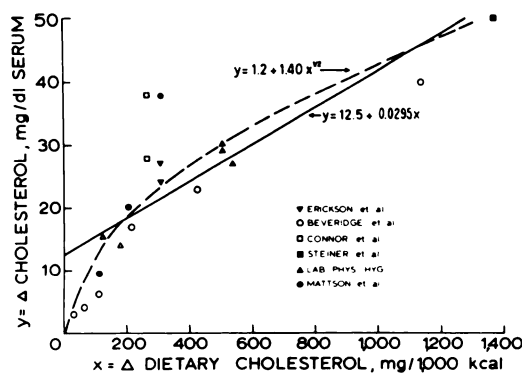


FIG. 1. Change in serum cholesterol in response to change in dietary cholesterol. Mean changes in 19 sets of experiments in which dietary cholesterol was the only variable. The lines shown depict the least squares regression with change in dietary cholesterol as such and again with the square root of change in dietary cholesterol.

average rise of only 28 mg/dl. In any case, all the data of Fig. 1 were subjected to linear regression analysis with the result:

$$\Delta\text{Chol, mg/dl} = 12.5 + 0.0295 \Delta\text{diet chol}$$

where diet cholesterol is measured as milligrams/1,000 kcal. The large intercept, $a = 12.5$ mg/dl, is troublesome, of course. The correlation between the change in the blood and that in the diet is $r = 0.79$. Note that this result indicates a change of only 3 mg/dl in the serum for a dietary change of 100 mg cholesterol/1,000 kcal, one-fourth that estimated by Mattson et al.

With the same data of Fig. 1, the regression analysis was repeated, using the square root of the dietary cholesterol as the dietary variable. The result was $\Delta\text{Chol} = -1.2 + 1.40 \Delta\text{diet chol}^{1/2}$. The correlation is improved; $r = 0.87$. Omission of the two outliers mentioned above and solution with $n = 17$ gave for the correlation between dietary change and serum change, $r = 0.90$ when dietary cholesterol itself was used, $r = 0.95$ when the square root was used.

Mattson et al. (53) estimated, on the basis of their own experimental data, that 61% of the serum cholesterol reduction in the National Diet Heart Study (54) could be explained by the reduction of cholesterol in the diet. The facts of that study must be recalled. There were two diets under test, B and C, which differed in the emphasis on total fat and on polyunsaturated fatty acids versus saturates. Diet D was supposed to be the control, resembling the ordinary American diet eaten by the subjects before they changed to one or another of the experimental diets. However, the requirement of double-blind design resulted in diet D being quite different from the pre-experiment freely chosen diet of the participants. Compared with the pre-experiment base-line period, the "control" D diet provided a 30% decrease in exogenous cholesterol as well as a decrease in saturated and an increase in polyunsaturated fatty acids.

The men in the five cities who changed to B diet showed an average fall of 25.4 mg/dl in the serum over weeks 12 through 52 on that diet. On the C diet, the corresponding figure was a fall of 27.6; on the D Diet there was a fall of 6.5 mg/dl. The mean base-line dietary cholesterol was estimated to be 208 mg/1,000

kcal, SD = 51. On diets B, C, D, the means, respectively, were specified to be 131, 128, 145, the standard deviations being 33, 38, and 32. In other words, all three diets involved a similar reduction of cholesterol in the diet so whatever may have been the effect of the change in dietary cholesterol, it could not explain more than a trivial part of the observed difference in the serum cholesterol response to the different diets.

The estimate by Mattson et al. that 61% of the serum cholesterol change in the National Diet Heart Study could be attributed to the change in dietary cholesterol is simply the result of applying to that data their regression equation based on their zero cholesterol intake control and three points of different cholesterol intake. Their equation is

$$\Delta\text{Chol, mg/dl} = 1.60 + 0.118 (\Delta\text{diet chol, mg/1,000 kcal})$$

Application of that equation to the data in the preceding paragraph gives predictions for the change in serum cholesterol expected on the basis of dietary cholesterol change alone: decreases of 10.7, 11.0 and 9.0 mg/dl on diets B, C, and D, respectively. Hence the equation of Mattson et al. would predict that differences in the cholesterol in the three diets would produce a serum cholesterol reduction in the men on diet B, as compared with the men on the control diet D, of $10.7 - 9.0 = 1.7$ mg/dl. For the comparison of men on diet C with those on diet D, the corresponding predicted difference would be $11.0 - 9.0 = 2.0$ mg/dl. As noted above, the observed differences were 18.9 and 21.1. In other words, with this reasoning, dietary cholesterol accounted for almost 9% of the observed difference between the control diet and diets B and C. Actually, it was never proposed that the dietary information obtained in the National Diet Heart Study would be adequate for precise evaluation of the factors affecting the serum cholesterol level but such as they are, the data do not point to a major effect of dietary cholesterol.

It was not possible to include, in Fig. 1, data from the important study by Hegsted et al. (55) on patients in a mental hospital. Though several dosages of dietary cholesterol were used they were not compared in diets otherwise identical. Furthermore, only three trials involved dietary cholesterol intakes below 150 mg/day. The



apparent effect of cholesterol in the diet in the study by Hegsted et al. was estimated from the coefficient obtained by solution of the multiple regression equation:

$$\Delta\text{Chol, mg/dl} = a + b_1 \Delta S + b_2 \Delta M + b_3 \Delta P + b_4 \Delta D$$

where D is measured as hundreds of milligrams of cholesterol in the daily diet. In their material of 36 dietary periods the finding was that $b_4 = 6.5$ meaning that for each 100 mg of cholesterol difference in the daily diet, the expectation is a difference of 6.5 mg/dl serum. No estimate of the standard error of b_4 was published but guidance is provided by the results of the different multiple regressions with consideration given to various combinations of the dietary variables. Interestingly, there is no statistically significant difference between the coefficients of multiple correlation with and without attention to cholesterol in the diet. In other words, dietary cholesterol could be ignored in the data of Hegsted et al. without significant loss in the ability to predict serum cholesterol change from knowledge of the change in the dietary fatty acids.

Mention should be made here of our experiment ME, which will be reported in detail in due course. A basal diet containing less than 2 mg cholesterol per 1,000 kcal provided 100 g daily in contrasting fats, safflower oil versus a mixture of two-thirds palm oil and one-third coconut oil. Each of these fats was fed with and without 300 mg cholesterol dissolved in the oil, the subjects being 12 healthy young men who received the four diets in a crisscross pattern. On the relatively saturated fat diet, the mean serum cholesterol concentration was 9 mg/dl higher with 305 mg cholesterol in the daily diet than when the intake was only 5 mg daily. On the safflower oil diet, that large difference in dietary cholesterol intake produced a mean difference in the serum of 8 mg/dl.

In experiment ME and in all the data summarized in Fig. 1, the serum cholesterol changes were responses to the addition of cholesterol to diets with no or near zero cholesterol in them. When small to moderate amounts of cholesterol are added to such diets the serum response may be approximately in linear proportion to the dietary addition. With larger additions, the relationship clearly is not linear, perhaps, as suggested by Mattson et al. (53), because of a limited capacity to absorb it,

perhaps because the bodily synthesis of cholesterol is limited by a feed-back mechanism.

Limiting consideration to diets without large amounts of cholesterol in them, excluding the data on the two highest intakes in Fig. 1, and including the two sets of data from experiment ME, the least-squares solution for 19 sets of data is: $\Delta\text{Chol} = 6.3 + 0.054 \Delta D$, D being milligrams of dietary cholesterol per 1,000 kcal. The correlation coefficient is $r = 0.77$, standard error of estimate = 6.8 mg/dl, standard error of the slope = ± 0.0107 , so the 95% confidence limits are nearly 0.052 and 0.056. Solution of the equation with the same data but using the square root of dietary cholesterol shows a closer relation with $r = 0.83$, but the difference from the result without transforming the dietary cholesterol is not statistically significant. Accordingly, a difference of 100 mg cholesterol per 1,000 kcal corresponds to a maximum difference of cholesterol in the serum of approximately 6 mg/dl.

The possible practical meaning of the foregoing must be assessed in the light of what free-living people actually eat. The average diet of physically active American men providing approximately 3,000 kcal daily contains roughly 600 to 700 mg cholesterol, or an average of 220 mg/1,000 kcal. Suppose one egg yolk is added to the diet. This means an increase of almost 50% in dietary cholesterol; the combined experimental evidence says the average response would be an increase of 5 or 6 mg in serum cholesterol. This is based on the findings in 19 sets of experiments of which 12 were with liquid formula diets. In view of the studies with natural foods, which found no or little effect of dietary cholesterol (42–45), it seems probable that the foregoing is an overestimate of the effect of exogenous cholesterol in ordinary diets.

2S - P

We have already indicated something of the development of the multiple regression equation for relating the serum cholesterol response to specified changes in the fatty acids in the diet. A quarter of a century ago it seemed that fat is fat and that knowledge of the amount of total fat in the diet might be enough to allow a rough estimate of the average serum cholesterol concentration. That is still valid for changes in



the amount of fat in the diet as long as the composition of the fat is unchanged. Then it was accidentally found that diets high in some vegetable oils produced quite different cholesterol levels than observed with the same amount of animal fats in the diet. For a short time the origin of the fat, animal versus vegetable, was much discussed. Soon, however, it dawned that in a biochemical relationship, chemical composition rather than origin of the substance must be the controlling factor. So the first test was to see what could be explained by 1) considering the simplest chemical breakdown of the food fats, or 2) consideration of the fatty acids as saturated, monoenes and polyenes with each class possibly contributing separately to influence the cholesterol in the blood. That idea was, Reiser would have it, our "preconceived notion."

The test of that idea was made, with all the experimental data available, using the multiple regression equation:

$$\Delta\text{Chol, mg/dl} = b_1 \Delta S + b_2 \Delta M + b_3 \Delta P,$$

in which S, M, and P are the percentages of the total calories in the diet provided by glycerides of saturated, monoene, and polyunsaturated fatty acids. Data from an experiment on a group of men studied on each of two diets provided one set of values for ΔChol , ΔS , ΔM , and ΔP ; with many such sets of values the equation can be solved by the method of least-squares so as to find the values of b_1 , b_2 , and b_3 that would best predict ΔChol . The answers were $b_1 = 2.74$, $b_2 = 0$, $b_3 = -1.31$ (8).

Reiser says (p. 550): "It was assumed that diet cholesterol plays no role, that all saturated fatty acids have the same hypercholesteremic effects, that all polyunsaturates have the same hypocholesteremic effects . . . and that all persons respond alike." This is arrant nonsense. The only assumption was that whatever might be the effects of the three classes of fatty acids, those effects would be algebraically additive. No assumption was made about any kind of fatty acid being either hyper- or hypocholesteremic. No assumption was made about an effect or lack of effect of dietary cholesterol; it was merely hoped that disregard of that variable would not prevent a useful answer, a hope that had some basis in the fact that in the diets tested, the overall correlation between the

amounts of the three classes of fatty acids and the amount of cholesterol in the diet was small. As for all persons responding alike, Reiser seems unaware that we had many times reported, documented, and analyzed in detail the fact that individuals differ in their serum cholesterol levels even when the diet and all other variables are controlled as closely as possible (8, 56). That fact persuaded us always to experiment with groups of men and in comparing groups to make sure they were matched as to average serum levels on a common reference diet. Reiser's statement, "They also admit that the equation is only valid for groups of men," disguises the fact that from the outset we made it perfectly clear that we are dealing with averages.

Reiser's statement: "These authors could not find confirmation of their formula in comparison of natural and hydrogenated safflower seed oil," (p. 550) is completely contrary to the fact; Reiser either did not read the paper (28) or he chose to misrepresent it. The data given in that paper fully justify the statement about experiment K (p. 391 of (28)): "The result, indeed, corresponds closely with prediction with the equation . . ." In regard to experiment N: "Again this general result was predicted but it may be asked whether the magnitude of the difference corresponds to expectation. Computation with the prediction equation indicates that somewhat larger differences might have been expected from subsistence using diets having the same proportions of S and P fatty acids as in experiment N but with no unnatural isomers present" (p. 391, 392). In experiment N, comparing safflower with hydrogenated safflower oil, the mean predicted difference was 22 mg/dl; the observed difference was 25 mg/dl (SE = ± 4.4). In experiment N, comparing corn oil with hydrogenated corn oil, the predicted mean difference was 21 mg/dl; the observed value was 27 (SE = ± 3.9). These values are easily verified from the data in Tables 3, 6, 7 in the paper and application of the equation: $\Delta\text{Chol} = 1.3 (2\Delta S - \Delta P)$.

Reiser is at pains to create the impression that our regression equation fails to fit the facts when examined by others and anyway has no particular meaning. He says (p. 551): "Ahrens pointed out that other equations could just as well fit the data, using oleic acid values instead



of linoleic acid, for example.” The article by Ahrens et al. to which Reiser refers (57), published in 1958, was written before publication of the critical experiments (58) which showed that indeed it is not possible to arrive at the corresponding result by considering oleic instead of linoleic acid. Reiser made no mention of reference 58 and apparently made no effort to learn what may be Ahrens’ opinion now.

Hegsted and his colleagues should have priority to respond to Reiser’s report and attack on their long series of experiments in a mental hospital (55). However, it must be noted here that they too examined their data by means of regression analysis. Because in their experiment the diets were tested serially rather than in Latin-square crossovers suitable for comparing diets in pairs of contrasts, their regression equations are not in the same form as our own but at least it is possible to compare coefficients. In the first place, they confirmed the absence of significant effect of oleic acid, the cholesterol-raising power of the total mixed saturates and the opposite effect of the polyunsaturates. Their material produced coefficients of 2.32 for S, -1.46 for P, so $S/P = 1.6$ (ref. 55, Table 4). In the same terms we had found 2.6 for S, -1.3 for P, and $S/P = 2$. Their use of cocoa butter in many of their diets and large proportions of saturates with fewer than 12 carbons in several diets is ample explanation that they found a slightly smaller cholesterol-raising effect of the total saturates than we did.

Reiser made no mention of any of this nor did he inform the reader about the critical comparison of our regression equation with that of Hegsted et al. (13). When our equation was used to predict the findings of Hegsted and his colleagues, the agreement was good, $r = 0.92$, the root mean square error being 14.9 mg/dl. The reverse procedure, prediction of our findings with the equation of Hegsted et al. also gave impressive agreement with $r = 0.87$ but the root mean square error was 28 mg/dl. This large overall discrepancy is attributable to the fact the Hegsted assumed a simple linear relationship between serum and dietary cholesterol and applied a large coefficient for dietary cholesterol when, as shown in their own analysis, dietary cholesterol could have been ignored without significantly reducing the predictive

power of the equation. That fault in the equation of Hegsted et al. was spotlighted in the analysis of the data of the National Diet Heart Study (54).

Reiser (p. 548) has many complaints about the report of Thomasson et al. (59) on experiments in which Trappists were given diets in which, aside from three pieces of fruit and 50 g bread daily, the nutrients were in a liquid formula including semisynthetic fats. Reiser writes: “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.” Apparently Reiser is unhappy with the conclusions of Thomasson et al. that “the cholesterol level appeared to be strongly dependent on the type of dietary fat, which confirms the results obtained by other investigations. The administration of saturated fatty acids is attended by an increase, that of linoleic acid by a decrease (in spite of the low initial values), whereas oleic acid occupies an intermediate position in this respect,” (ref. 59, p. 634). Reiser made no mention of these conclusions.

Thomasson et al. also made an analysis of their data by multiple regression, a fact not mentioned by Reiser, and they wrote: “Although a comparison of the present equations with that of Keys would be incorrect (different endogenous and exogenous conditions and, moreover, use of change in fatty acid composition by Keys) both equations express that the cholesterol level is influenced in the order saturated, mono-unsaturated and di-unsaturated.” We agree that the conditions of the experiments on the Trappists and the form in which the data were obtained make comparison difficult but it is interesting that the multiple regression solution with the data on the men in the experiment of Thomasson et al. gave a positive coefficient for S, a negative coefficient for P, and a ratio of the coefficients, $S/P = 1.7$.

Reiser refers at the start (p.524) and again at the end (p. 551) of his piece to a recent paper of ours on diets of different fatty acid composition that produce identical serum cholesterol levels (60). About that paper he wrote: “A recent paper by Grande et al. is an example of how one can be misled by a questionable major premise (3)” (p. 551). The idea that

exchanging fats with $2S - P = 0$ should have no effect is not in the least a "questionable major premise;" it is simply the logical result of the multiple regression equation which is an analytical finding, a fact Reiser seems incapable of comprehending. Because we were well aware that regression and correlation analyses have limitations in establishing cause and effect, we decided that a critical experiment was desirable. A logical conclusion from our equation would be that the serum cholesterol level should not change when dietary fats are altered in amount or kind as long as the proportion of total calories provided by polyunsaturated fatty acids remained constant at twice the proportion of calories provided by saturated fatty acids with 12 through 16 carbons. Therefore, diets with different amounts of different fats were devised that had the common property of having in them double the amount of polyunsaturates (actually linoleic acid) as compared with the saturates with 12 through 16 carbons. The experiments nicely confirmed the prediction from the equation. Reiser's total failure to understand the logic and the results of those experiments is the kindest explanation for his conclusion about that study: "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 . . . (p. 551).

Though Reiser failed to comprehend what those experiments were all about, he went on to write that the results were "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." As authority he cited Hegsted et al. (55) and Filer et al. (41). Dr. Hegsted may respond but here we must insist that Reiser's attribution to Hegsted et al. is a crude distortion. In the multiple regression analysis, Hegsted found a larger coefficient for myristic than for palmitic acid, opposite to Reiser's representation, but in a subsequent critical test reported as an addendum to the paper, no difference between the effect of the two fatty acids could be seen. We are not aware that any responsible investigator in this field has ever held that "lauric acid has no effect." What Hegsted et al. wrote was that a solution with 8 dietary variables "is not a significantly better fit than equation 3 and it may be

concluded that it is unlikely that consideration of S_{10} , S_{12} , S_{18} and M assist in predicting serum cholesterol *after* S_{14} , S_{16} , P and C are considered" (ref. 55, p. 288; their italics). As for the other authority, we repeat that the paper by Filer et al. (41) has nothing whatever to do with serum cholesterol. Reiser's statement that Filer et al. came to the conclusions he attributed to them is pure invention.

The final "telling arguments"

Just before Reiser starts his undocumented generalizations under the heading "Comments," he writes triumphantly: "The most ardent advocates of the saturated fat theory cannot agree. The Minnesota group concludes 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 believes 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?" This is gross fabrication. We do not and never did conclude that "stearic acid can neutralize palmitic in cocoa butter." As for the Harvard group's position, they should respond. Above, we have commented on lauric acid in their experiments at the mental hospital. We have no idea where Reiser got the idea that the Harvard group "support palmitic except in some fats such as cocoa butter and olive, they grant that palmitic is neutral." We suspect, again, that this is simply a fabrication.

Reiser, under "Comments," leads off by stating that "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 or cocoa butter. How is it possible, therefore, to attribute the phenomenon of hypercholesterolemia to saturated fatty acids?" This statement epitomizes Reiser's whole article. It is a tissue of untruths. It is not agreed that "acids of 12 carbon atoms or less are not involved." It has been shown experimentally (61), and everyone agrees, that

saturated fatty acids of 10 carbon atoms or fewer do not raise the serum cholesterol level. The explanation is the well-documented fact that such short- and medium-chain saturates are digested, absorbed, and metabolized in a quite different way from the fatty acids with 12 or more carbons in the chain (62, 63). Why stearic acid is also relatively neutral is unknown but there is no disagreement that it is neutral. It is not agreed, nor even proposed by anyone (except Reiser himself, perhaps, on the basis of his imagination) that "palmitic is not effective in such oils as olive and cocoa butter."

Reiser's remarks about failure to consider mechanisms of the effects of the fatty acids on serum cholesterol include a complaint that there have been no efforts to explain how "exogenous saturated fat, as distinct from endogenous fat of the same fatty acid composition, has the opposite effect." Apparently, Reiser is in possession of evidence to this curious situation which he does not share with the reader. We propose that until proved otherwise, this is only one more of Reiser's "inventions."

The mechanisms whereby dietary fatty acids affect the metabolism of cholesterol and its concentration in the serum are complex (64, 65) and theories are still controversial (66), but the reality of the effects is not. The same is true of the effects of dietary cholesterol; research continues to discover how it works (67, 68). Reiser's position is that the fatty acids do not affect serum cholesterol, or at least the saturated ones do not, but anyway if they do, they act by influencing the absorption of cholesterol or the action of phytosterols. We have shown how unlikely it is that such hypothetical actions could explain more than a small part of the observed effects of changing fats in the diet, but this is not the issue. The question is: Do these saturated fatty acids in the diet cause a rise in serum cholesterol? That question is no longer debatable. How the result is brought about will be known in due course; the question is the subject of intense study in a number of research centers.

Later (p. 552) Reiser states: "The half-hearted efforts during the 1950's to dissociate the two constituents of animal fat resulted in exoneration of the cholesterol. Although it has gradually become acknowledged that cholesterol is the more responsible partner, satu-

rated fat is still blamed in loose use of terms and remains as the co-villain of the drama in the minds of the undiscerning." Workers in this field, and Reiser is not nor has he been a worker in the field, will properly rise in wrath at Reiser's use of descriptives, "half-hearted," "undiscerning." And it is not true that it is "acknowledged that cholesterol is the more responsible partner;" at most, it is agreed that dietary cholesterol can have some effect.

Reiser pompously states: "Perhaps the key experimental oversights in these studies have been the failure to firmly establish the serum concentrations on one diet before changing to another, and proper interpretation of the curves relating serum cholesterol to diet changes." There have been some experiments of too short duration to show the full effects of a change in the diet. In no case we know about was the direction of change mistaken because of this. As noted earlier, Reiser was happy to cite the briefest of dietary experiments when they could be made to appear to support his views. He completely ignores the unanimous finding in dozens of studies that all, or nearly all, of the dietary fat-serum cholesterol response of man is exhibited in 2 or at most 3 weeks. The fact is that almost all of the evidence for the "saturated fat theory," as Reiser calls it, was obtained with dietary periods of that length or longer. As for the "curves," that word again reflects Reiser's failure to understand that, after a couple of weeks, any "curves" reflect laboratory or seasonal trends that are easily compensated for by using our standing cross-over design of dietary change.

Near the end of his article (p. 552), Reiser adverts again to one of his favorite ideas, "lack of appreciation of the wide fluctuations that can normally occur." Obviously, Reiser has no comprehension of the way statistical theory deals with such "fluctuations." In all of the major studies we know about, including our own extending over many experiments in a span of over 20 years, such fluctuations were fully compensated. Reiser's citation (69) of our old and long dead friend, "Chuck" Wilkinson, is entirely beside the point. Wilkinson was objecting to crediting a positive effect of sitosterol in the diet without considering the variations in serum cholesterol when sitosterol was not administered. We agree 18 years later, as we did at the time of Wilkinson's publication, but that

does not change the fact that such "normal variations" have been fully allowed in all of the major studies which Reiser attacks.

The final point that Reiser says causes "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" (p. 552). We do not understand what is meant by "standard people," but then we fail to understand Reiser, period. ❏

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