Quantitative Effects of Dietary Fat on Serum Cholesterol in Man

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Although many studies demonstrate that changes in the serum cholesterol level may be induced by modifying the amount or composition of dietary fat, the causal factors in oils and fats have not been clearly identified. Major emphasis has been placed upon the polyunsaturated fatty acids, both in the scientific and the popular press. Not all the evidence, however, supports this presumed primary role of polyunsaturated fatty acids nor will the conclusions of this paper.

The principal conclusions regarding the relationship between various fat components and the level of serum cholesterol may be summarized as follows:

1) Ahrens et al. demonstrated a rough inverse relationship between the serum cholesterol level and the iodine number (total degree of unsaturation) of various oils. This conclusion implies that monounsaturated acids are half as effective as the diene, linoleic acid, the primary polyunsaturated acid in vegetable oils. The data seem to demonstrate that minimal levels of serum cholesterol were achieved with oils with an iodine number of about 100, and that more highly unsaturated oils were not more effective. Since all fats were fed at the same fat level, the effects of varying the amount of dietary fat were not assessed. More recently Gunning et al. suggested that the square root of the iodine number rather than the iodine number itself is inversely related to the level of serum cholesterol.

2) Keys et al. in the most comprehensive studies yet reported fed groups of men diets containing various kinds and amounts of fats. From changes in serum cholesterol induced with the various diets, the multiple regression equation

\[ \text{cholesterol} = 2.76 \Delta S + 0.05 \Delta M - 1.33 \Delta P - 1.68 \]

was derived in which \( \Delta S, \Delta M, \) and \( \Delta P \) are the changes in the per cent of dietary calories derived from saturated, mono- and polyunsaturated fatty acids. Since the coefficient of \( \Delta M \) and the constant 1.68 were not statistically significant these were eliminated and the predictive equation became

\[ \Delta \text{cholesterol} = 2.74 \Delta S - 1.31 \Delta P \]

These workers concluded that the equation demonstrated that "saturated fatty acids have, per gramme, about twice as much effect on serum cholesterol as do the poly-ethenoids which, moreover, act in the opposite direction. Whether the mono-ethenoids (essentially oleic acid here) have any effect is uncertain, but if there is an effect it is certainly small." In later reports, concentrates of oleic acid were fed and the apparent lack of an effect was confirmed. Modifications in the predictive equation to account for differences in response in various degrees of hypercholesterolemia were also introduced.

3) Kinsell et al. emphasized the role of the polyunsaturated or essential fatty acids al-
TABLE I
Food Pattern of 2,600 Calorie Diet

<table>
<thead>
<tr>
<th>Food</th>
<th>gm./day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits, vegetables, potatoes</td>
<td>730</td>
</tr>
<tr>
<td>Eggs</td>
<td>56</td>
</tr>
<tr>
<td>Lean meat, fish, cheese</td>
<td>204</td>
</tr>
<tr>
<td>Skim milk powder</td>
<td>71</td>
</tr>
<tr>
<td>Bread</td>
<td>49</td>
</tr>
<tr>
<td>Other cereals</td>
<td>125</td>
</tr>
<tr>
<td>Flour</td>
<td>54</td>
</tr>
<tr>
<td>Honey, syrup, jelly</td>
<td>27</td>
</tr>
<tr>
<td>Sugar</td>
<td>62</td>
</tr>
<tr>
<td>Soups</td>
<td>88</td>
</tr>
<tr>
<td>Test oil</td>
<td>93</td>
</tr>
</tbody>
</table>

though quantitative data have not been obtained relating response to dose. Ahrens and his associates have demonstrated a cholesterol lowering effect of the fish oils containing large amounts of polyunsaturated acids which have little essential fatty acid activity for the rat, but Keys et al. found that highly unsaturated fish oils do not appear to be more hypocholesterolemic than oils containing primarily linoleic acid. Moreover the work of McOsker et al. and Erickson et al., in which oils subjected to varying degrees of hydrogenation were studied, indicated no significant effect on serum cholesterol even with substantial changes in the polyunsaturated fatty acid content.

(4) Although the role of dietary cholesterol was generally discounted several years ago, practically all recent investigations demonstrate an effect.

(5) Numerous factors other than the dietary fat are known to influence serum cholesterol levels. These include the phytosterols, the kind of dietary carbohydrate, dietary protein and probably other factors. It should be recognized that the role such factors play may be partially dependent upon the composition of the dietary fat or that the response to dietary fat may be partially dependent upon factors such as those mentioned. In general, quantitative data indicating such relationships are lacking.

Undoubtedly, the composition of the usual diet in the United States is now being changed by the emphasis upon the role of dietary fat in atherosclerotic heart disease. It would seem mandatory, in view of the implications for public health and the economic impact on the food industries, that the changes made be those which are most effective. This cannot be accomplished until the causal factors are clearly defined and their effects quantitatively assessed.

The data to be presented consist of the responses in serum cholesterol of groups of men, ten men per group, who were fed a low fat diet to which various test fats were added. In some test periods, the level of dietary cholesterol was also varied. The data have been analyzed to determine the serum cholesterol response to various dietary fatty acids and to dietary cholesterol. An unusually strong feature of these studies is that the comparisons have been made with the same men throughout the study.

MATERIALS AND METHODS

The diets used in these studies were based upon a mixture of low fat foods which were calculated to supply a minimal amount of fat, to provide 15 to 16 per cent of the calories as protein, to provide a relatively constant daily level of cholesterol intake and to allow menu planning that would represent a variety of foods which could be easily standardized and reproduced. To this pattern of foods, the oils to be tested were added at appropriate levels, and additional calories as needed were supplied by carbohydrate sources—starch, sugars, potatoes, cereals and breads.

The diets were designed to supply sufficient calories to maintain the weight of the subjects and to hold the percentage of calories supplied from fat and protein approximately constant for all the men in any particular test. It was necessary, therefore, to devise diets yielding approximately 2,200, 2,600 and 3,000 calories per day. These levels were determined by measurement of the food eaten by the subjects before the studies began, and by observing the weights of the men throughout the experiment period. The foods used in the 2,600 calorie, 38 per cent fat level diet are listed in Table 1. Food portions were proportionally increased or decreased to yield the other calorie and fat levels.

A seven day menu cycle was planned which was repeated weekly for the entire experiment. Meats, the principal source of nonfat test, were carefully selected and trimmed and purchased in as large quantities as possible. The test oils were used primarily by incorporating them into recipes for many products such as waffles, muffins, cakes, cookies, pie crust, biscuits, salad dressings and spreads for bread.
This necessitated considerable experimental work to obtain satisfactory products with certain oils. Filled milk and ice creams, prepared from nonfat milk and the appropriate oil, were used in all tests.

Several weekly composites of the diets were prepared during the study. These were homogenized and aliquots extracted according to the A.O.A.C. procedure. The total extract was taken to drynes, redissolved in ligoine and the weight of fat determined. The 2,600 calorie diet plan as described in Table I, but without the "test" fat, contained 10.3 gm. of fat according to this procedure, whereas calculations from food tables indicated a content of 20 gm. (Table II). Based upon the analytical value, the diets finally developed contained approximately 22 and 38 per cent of the calories as fat rather than the 25 and 40 per cent levels actually planned. The fatty acid composition of the extract was determined by gas chromatography after methylation of the fatty acids. Cholesterol determinations on the crude extract indicated 306 mg. per day whereas a value of 555 mg. was calculated from published figures. It is recognized that the colorimetric procedure for cholesterol is not entirely specific and various plant sterols and other products undoubtedly influence the results obtained to some extent. Values calculated from the literature are similarly untrustworthy. The exact cholesterol content is therefore unknown. However, the amount of cholesterol supplied can be considered essentially constant in all test periods and all control periods.

The "control" period diet was designed to approximate a usual American diet. It was the same as the experimental diet, except that milk and ice cream contained butterfat and the oils and fats used in food preparation and service were margarine, mayonnaise, lard and high P:S ratio vegetable shortenings. Analysis of this diet at the 2,600 calorie level indicated a content of 331 mg. cholesterol per day as compared to a calculated value of 660 mg.

The subjects were selected from a rather large group in the mental institution. Most of the men were chronic schizophrenic patients without evidence of physical disease and were from thirty-eight to fifty-seven years of age. Those with serum cholesterol values above 300 or lower than 200 mg. per cent were excluded. The final selection was based primarily upon the probability that they would be available and cooperative over a long period of time.

While all the men were housed and ate in an isolated ward which included a recreation room in addition to the kitchen and dining facilities, they continued their usual supervised activities in the institution. The purposes of the study and the need for adherence to the diet were continually stressed to attendants and subjects, and failure to comply meant removal from the study.

Three men were replaced after the third month and the group has been nearly constant since that time. Their desire to remain on the study undoubtedly reflects the quality of the food supplied and the continuous attention they receive. Opportunities for obtaining additional foods are very limited and infractions of the rules have been minimal. The uniformity of the data obtained support this conclusion.

Each test period was of four weeks' duration. Blood samples were obtained before breakfast in heparinized capillary tubes by ear puncture two weeks after the start of each diet and again at three and one-half and four weeks. Samples were analyzed for total serum cholesterol, beta-lipoprotein cholesterol by a micro modification in which cholesterol is measured in the precipitate with a sulfated polygalacturonic acid, total fatty acids and lipid phosphorus. From these values, total serum triglyceride was calculated by subtracting cholesterol ester fatty acid (assuming cholesterol to be 70 per cent esterified) and phospholipid fatty acids from the total fatty acids. In accordance with previous experience, the major cholesterol response is obtained within the first two weeks. The average of the two terminal values at three and one-half and four weeks was used in all calculations.

During the first year of the study the men received the "test" and "control" diets in alternating four week periods. During the first months, the serum cholesterol levels resulting from the control diet gradually fell. Change in serum cholesterol (Δ cholesterol) was calculated by comparing the mean value at the termination of the test diet with the average of the values on the control diets immediately preceding and following the test period.
After the first year, the values on the control diet stabilized and the control diet was given only at widely spaced intervals. The fatty acid and cholesterol content of the experimental diets, arranged in the order in which they were fed, and the \( \Delta \) cholesterol values are listed in Table III.

Safflower oil, olive oil and coconut oil supply the largest amounts of polyunsaturated fatty acid (linoleic acid), monounsaturated (oleic acid) and saturated acids, respectively, that can be obtained with the ordinarily available edible oils. Coconut oil, although highly saturated, is well utilized because it contains relatively large amounts of lauric and myristic acids. These acids are not major constituents of many edible oils and the possibility was considered that these saturated acids may have properties not typical of other saturated acids. Triglycerides of longer chain fatty acids are not well utilized. Cocoa butter with over 60 per cent of palmitic and stearic acid contains the maximal amount which can be incorporated into a fat without the formation of poorly utilized saturated triglycerides. These factors determined the oils chosen for tests during the first year of the experiment which included the maximal spread in saturated (S), monounsaturated (M) and polyunsaturated (P) acids which could be achieved readily. Each of these oils as well as a mixture which provided equal amounts of saturated, monounsaturated and polyunsaturated acids were tested at two levels of dietary fat providing 22 and 38 per cent of the calories as fat. Tests with safflower oil and, later, with olive oil, were repeated.

Butterfat is known to be more hypercholesterolemic than most oils. Studies during the second year included a variety of mixtures of butterfat and other oils to study the influence of the latter upon hypercholesterolemia induced by butterfat. All of these tests were made with fat providing 38 per cent of the total calories.

The addition of butterfat to the diet and substitution of part of the butterfat by other oils resulted in substantial changes in the level of dietary cholesterol. Studies were therefore made with safflower, olive and coconut oil in which the dietary cholesterol was deliberately varied. A relatively low cholesterol diet was achieved by eliminating egg yolk from the diet. The higher cholesterol diet was achieved by adding additional egg yolk. The fatty acid and cholesterol content of the diets after these modifications are also given in Table III.

A total of thirty-six different test diets have been studied. All the data in Table III were coded for machine computation utilizing the IBM 7094 at the Harvard Computing Center. All possible equations for predicting changes in serum cholesterol using each variable singly and in all combinations were determined. With eight dietary variables, the various saturated acids, the mono- and polyunsaturated acids and dietary cholesterol, there are 256 equations. The data were also coded with the fatty acids expressed as percentage of the dietary fat, rather than as percentage of total calories, and the computations repeated.

**RESULTS**

The mean serum cholesterol of the group of men finally selected was 225 mg. per cent while consuming the house diet. When the diet was changed to the "control" diet, the level rose to 250 mg. per cent. In the following six months, the values obtained during the control periods gradually fell to approximately 220 mg. per cent. They remained essentially stable and at this level while the men were on the control diet during the following periods. The causes of these changes are unknown and represent one of the limitations of studies of this kind, i.e., even though the diet is as carefully controlled as possible, the level of serum cholesterol is not strictly reproducible. As has been explained, the change in serum cholesterol (\( \Delta \) cholesterol) was calculated by subtracting the mean values of each man in the immediately preceding and succeeding control periods from the values obtained while they were on the experimental diet.

The mean \( \Delta \) cholesterol values for each test diet are given in Table III. The standard errors of these values are of the order of 5 or 6 mg. per cent and it may be concluded that the values obtained are expected to be reproducible within about 10 mg. per cent. Greater accuracy could be achieved only with larger groups of men.

We were unable to demonstrate differences in the response of serum cholesterol in men with different inherent levels of serum cholesterol. That is, men who had higher levels of serum cholesterol did not show greater changes than those with lower levels. This is probably because the men were selected to give a relatively narrow range of values at the beginning of the study. All results are recorded as
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### TABLE III
Composition of the Diets Studied and the Changes Induced in Serum Cholesterol Levels

<table>
<thead>
<tr>
<th>Period</th>
<th>Diet</th>
<th>No.</th>
<th>% Total Fat Calories</th>
<th>Saturated Fatty Acids</th>
<th>Dietary Cholesterol (mg/day)</th>
<th>Δ Serum Cholesterol (mg.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total S</td>
<td>Total M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&lt;12:0)</td>
<td>(12:0)</td>
<td>(14:0)</td>
<td>(16:0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>38</td>
<td>0.47</td>
<td>0.25</td>
<td>0.96</td>
<td>5.65</td>
</tr>
<tr>
<td>Group I</td>
<td>BF-Cotton</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Safflower 70-50</td>
<td>9</td>
<td>38</td>
<td>1.30</td>
<td>0.66</td>
<td>2.20</td>
</tr>
<tr>
<td>2</td>
<td>BF-Corn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BF-Safflower 85-15</td>
<td>9</td>
<td>38</td>
<td>2.21</td>
<td>1.12</td>
<td>3.46</td>
</tr>
<tr>
<td>4</td>
<td>BF-Olive 70-30</td>
<td>9</td>
<td>38</td>
<td>1.82</td>
<td>0.92</td>
<td>2.83</td>
</tr>
<tr>
<td>5</td>
<td>BF-MCT 50-50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Safflower low cholesterol</td>
<td>10</td>
<td>37</td>
<td>0.00</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>7</td>
<td>Safflower high cholesterol</td>
<td>10</td>
<td>40</td>
<td>4.29</td>
<td>14.91</td>
<td>5.93</td>
</tr>
<tr>
<td>8</td>
<td>Olive high cholesterol</td>
<td>10</td>
<td>22</td>
<td>0.00</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>Group II</td>
<td>Safflower 85-15</td>
<td>9</td>
<td>38</td>
<td>2.60</td>
<td>1.31</td>
<td>4.00</td>
</tr>
<tr>
<td>19</td>
<td>Safflower 50-50</td>
<td>9</td>
<td>38</td>
<td>1.30</td>
<td>0.66</td>
<td>2.05</td>
</tr>
<tr>
<td>20</td>
<td>Safflower 70-30</td>
<td>9</td>
<td>38</td>
<td>1.82</td>
<td>0.92</td>
<td>2.93</td>
</tr>
<tr>
<td>21</td>
<td>Safflower 85-15</td>
<td>9</td>
<td>38</td>
<td>2.21</td>
<td>1.12</td>
<td>3.41</td>
</tr>
<tr>
<td>22</td>
<td>BF-Olive 85-15</td>
<td>9</td>
<td>38</td>
<td>2.21</td>
<td>1.12</td>
<td>3.90</td>
</tr>
<tr>
<td>23</td>
<td>Olive high cholesterol</td>
<td>10</td>
<td>40</td>
<td>0.00</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>24</td>
<td>Olive low cholesterol</td>
<td>10</td>
<td>37</td>
<td>4.55</td>
<td>15.80</td>
<td>6.27</td>
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<tr>
<td>25</td>
<td>Olive high cholesterol</td>
<td>10</td>
<td>38</td>
<td>0.00</td>
<td>0.01</td>
<td>0.10</td>
</tr>
</tbody>
</table>
changes in milligrams per cent rather than as per cent of the serum cholesterol level.

The cholesterol level of the beta lipoproteins was found to parallel changes in total cholesterol levels. The values, therefore, are not reported. Changes in total serum triglycerides and serum phospholipids were found to be in the same direction as the serum cholesterol levels. However, the changes were less consistent than those of the serum cholesterol and the standard errors of the differences were large. They did not appear to contribute usefully in this study and are also not reported at this time.

As shown in Table III, the dietary fatty acid components were divided into five classes of saturated fatty acids (S10-518), the monounsaturated acids (M), and the polyunsaturated acids (P). The monounsaturated acids consisted almost entirely of oleic acid with small amounts of palmitoleic acid. The polyunsaturated acid in most oils was linoleic acid. Fish oil and butterfat contained a variety of other polyunsaturated acids. The change in intake of each of these classes of fatty acids as well as dietary cholesterol was determined by comparing the intake to that obtained from the control diet.

The complete regression equation, including all the fatty acid classes and dietary cholesterol, would have the form

$$\Delta \text{ch} = a \Delta S_0 + b \Delta S_4 + c \Delta S_4 + d \Delta S_6 + e \Delta S_8 + f \Delta M + g \Delta P + h \Delta C + \text{constant} \quad (1)$$

The problem is to determine the significance of the regression coefficients a to h. In the discussion which follows, the notation in Table III is utilized in the equations and all computations have been made based upon differences in the values in the control diet and the experimental diet. Thus, the equations appear as

$$\Delta \text{ch} = a S_0 + b S_4 \ldots$$

the \(\Delta\) signs which appear in equation (1) being omitted.

The multiple correlation coefficient, \(R\), is the statistical parameter most commonly used to indicate the ability of the regression equation to predict the experimental data. \(R^2\) is more easily interpreted since this is the per cent of the total variance in serum cholesterol which is accounted for by the regression equation. The standard deviation from regression is the measure of the deviation about the multiple regression line.

### Calculations Based upon Fatty Acids Expressed as Per Cent of Total Calories

In the calculations first made the role of the total saturated, S, monounsaturated, M, and polyunsaturated, P, fatty acids and dietary cholesterol, C, were assessed. The multiple regression equation including these variables is

$$\Delta \text{ch} = 2.32 S + 0.32 M - 1.46 P + 6.51 C + 0.83 \quad (2)$$

As observed by Keys et al., the regression coefficient for S is large and positive indicating that increases in S raise serum cholesterol. P has a negative coefficient and the coefficient of M is relatively small. The coefficient for changes in dietary cholesterol is 6.51, suggesting an average increase in serum cholesterol of 6.5 mg. per cent when the dietary cholesterol is raised 100 mg. per day. As shown in the first line of Table IV the correlation coefficient is high, 0.937, and approximately 88 per cent \((R^2 = 0.879)\) of the total observed variation in serum cholesterol is explained by this equation. The standard deviation about the regression line is 12.98 mg. per cent.

The succeeding lines in Table IV contain the regression coefficients for the equations of best fit as variables are deleted. When M is not considered, line 2, the fit is substantially the same and \(R = 0.936\) (Fig. 1). However, when dietary cholesterol is omitted the ability of the equation to account for the observed changes falls appreciably \((p < 0.001)\). Dietary cholesterol is therefore a significant variable. Finally, with the omission of P, the predictive ability falls still further. Nevertheless it should be emphasized that changes in the saturated acids alone account for 72 per cent of the total variation in serum cholesterol observed. Although the regression coefficient for P is approximately as large as that of S in line 3, the addition of P to the equation only increases
the predictive ability of the equations 8 per cent, i.e., from 72 to 80 per cent.

In the next step in the analyses the saturated acids were divided into the classes indicated in Table III. From the many regression equations obtained, only a few of particular interest can be presented. The most important, however, is the equation

$$\Delta \text{ch} = 8.45 \text{S}_{14} + 2.12 \text{S}_{16} - 1.87 \text{P} + 5.64 \text{C} - 0.24 \quad (3)$$

As shown in Figure 2 and in Table V, the correlation coefficient with this equation involving only four of the dietary variables is 0.951 and 90 per cent of the total variation in serum cholesterol is explained by this equation. The fit is substantially better than any of the equations shown in Table IV. In lines 2 to 5, the effects of deleting each of these variables is shown. It will be seen that the elimination of palmitic acid, $\text{S}_{16}$, has the least effect, the correlation coefficient falling only to 0.941 (line 4). Variance analyses, however, demonstrate this to be a significant change ($p = 0.02$). The greatest change is observed when $\text{S}_{14}$, myristic acid, is deleted. Deletion of polyunsaturates or dietary cholesterol have lesser but significant effects. Line 6 shows that changes in myristic...
TABLE V

Characteristics of Regression Equations When the Fatty Acids Are Expressed as Per Cent of Calories

<table>
<thead>
<tr>
<th>Line</th>
<th>Variable</th>
<th>Constant</th>
<th>$R^2$</th>
<th>$R^{*}$</th>
<th>S.D. from Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_{10}$* (% cal.)</td>
<td>$S_{12}$† (% cal.)</td>
<td>$P_{12}$‡ (% cal.)</td>
<td>$C_{5}$§ (mg. X 100)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.45</td>
<td>2.12</td>
<td>-1.87</td>
<td>5.64</td>
<td>-6.24</td>
</tr>
<tr>
<td>2</td>
<td>9.50</td>
<td>3.24</td>
<td>-1.63</td>
<td>...</td>
<td>-1.73</td>
</tr>
<tr>
<td>3</td>
<td>11.58</td>
<td>3.66</td>
<td>...</td>
<td>3.02</td>
<td>-4.80</td>
</tr>
<tr>
<td>4</td>
<td>8.78</td>
<td>...</td>
<td>-2.10</td>
<td>7.50</td>
<td>-8.02</td>
</tr>
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<td>5</td>
<td>...</td>
<td>2.80</td>
<td>-2.86</td>
<td>9.28</td>
<td>-2.51</td>
</tr>
<tr>
<td>6</td>
<td>14.22</td>
<td>...</td>
<td>...</td>
<td>3.25</td>
<td>0.830</td>
</tr>
</tbody>
</table>

* Myristic acid.
† Palmitic acid.
‡ All polyunsaturated acids.
§ Dietary cholesterol was expressed in units of 100 mg.
* Multiple regression coefficient.
† $R^2$ is a measure of the total variance explained by the regression equation.

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.

It is most important to note that the regression coefficients may change appreciably for all variables depending upon the particular variables included in the equation. For example, the coefficient for cholesterol is tripled in line 5 as compared to line 3 and the coefficient of $P$ is nearly twice as high in line 5 as compared to line 2. This is due to the fact that the variables are not independent and that the coefficients are intercorrelated. Snedecor stresses the fact that "statements made about the predictive value of a variable are not unique; they depend upon the other variables being used in the regression."

Calculations Based upon Fatty Acids Expressed as Per Cent of Dietary Fat

Calculations similar to those shown in Tables iv and v were also made with the dietary fatty acids expressed as per cent of total dietary fat. Generally speaking, the results are quite similar. The coefficients for the important regression equations are shown in Table vi. Analysis of variance demonstrates that each of these variables contributes significantly to the ability of the equation to account for changes in the serum cholesterol. It is most important, how-
Quantitative Effects of Dietary Fat

TABLE VI
Characteristics of Regression Equations When the Fatty Acids Are Expressed as Per Cent of Dietary Fat*

<table>
<thead>
<tr>
<th>Line</th>
<th>Variable</th>
<th>Constant</th>
<th>R2</th>
<th>S.D. from Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S4 (% of fat)</td>
<td>S6 (% of fat)</td>
<td>P (% of fat)</td>
<td>C (mg. X 100)</td>
</tr>
<tr>
<td>1</td>
<td>3.27</td>
<td>0.89</td>
<td>-0.61</td>
<td>5.88</td>
</tr>
<tr>
<td>2</td>
<td>3.68</td>
<td>1.23</td>
<td>-0.56</td>
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<td>3</td>
<td>4.42</td>
<td>1.73</td>
<td>4.94</td>
<td>6.94</td>
</tr>
<tr>
<td>4</td>
<td>3.14</td>
<td>...</td>
<td>-0.75</td>
<td>9.14</td>
</tr>
<tr>
<td>5</td>
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* See footnotes of Table v for explanation of column headings.

ever, to note that the equation given in line 1 of Table vi and Figure 4

\[ \Delta \text{ch} = 3.27 S_4 + 0.89 S_6 - 0.61 P + 5.88 C - 6.7 \] (5)

is actually slightly superior to equation 3 given in line 1 of Table v. Thus with these data, consideration of only the proportions of fatty acids in the dietary fat without consideration of the amount does not impair the ability to predict serum cholesterol change.

Lines 6 to 11 in Table vi indicate the ability of individual and some pairs of variables to predict changes in serum cholesterol. The relatively high correlations with myristic acid and polyunsaturated acids are again apparent as is the limited effect of palmitic acid. The large changes in the size of the regression coefficients depending upon the variables included in the equation should also be noted.

COMMENTS

The major difficulty in studies of this kind in arriving at firm conclusions as to the physiologic role of dietary components should be clearly understood. When naturally available oils are used, the dietary variables are correlated to a greater or lesser extent. Much of the same information is imparted regardless of which variable is considered and practically any variable may show considerable correlation with change in serum cholesterol. But these effects are not additive. For example, in Table vi, line 6, the per cent myristic acid is shown to correlate highly, \( R = 0.817 \). This variable has the highest correlation singly of any variable we have considered. However, P alone is also highly correlated (line 10, \( R = 0.780 \)), whereas palmitic acid is correlated to a much lesser degree (line 9, \( R = 0.538 \)). Nevertheless, the combination of myristic and

FIG. 4. Scatter diagram showing the correlation between the values found and those predicted by the regression equation. \( S_4, S_6, \) and P are expressed as per cent of dietary fat. Compare to Figure 2 in which these components were expressed as per cent of dietary calories.
palmitic acid (line 7) provides nearly as good a fit for the data as does the combination of myristic and P together (line 8).

Further, as has already been indicated, the size of the regression coefficients depends upon the other variables included in the equation. Snedecor32 has explained why this is true. These equations are primarily descriptive of the information from which they are derived. Should new variables be included, such as a dietary carbohydrate component, the regression coefficients for the fatty acids might be expected to change. It is somewhat hazardous to attach as much functional significance to the regression equations as Keys et al.3 have done.

It is surprising indeed to find that myristic acid appears to be a most important variable in influencing serum cholesterol. The saturated acids containing 10 carbons and less are known to have essentially no hypercholesterolemic action33,34 and this was confirmed in period 13 (Table III) when a 50 to 50 mixture of butterfat and medium-chain triglycerides was fed. The shorter chain fatty acids are known to be absorbed primarily via the portal system whereas the longer chain acids enter the lymphatic system. If dietary fats influence cholesterol absorption, it might have been suspected that the distinction between effective and noneffective fatty acids would occur between the C10 and C12 acids since the latter, lauric acid, appears to be primarily absorbed via the lymph. We found no evidence, however, that lauric acid is a significant variable in these studies.

Another factor which must be considered is the degree of variation obtained in the dietary component under study. Although a substance may influence serum cholesterol, this will not be evident unless it is varied in the experiment. Conversely, a component which is varied greatly in the experimental design has a better opportunity to manifest its influence. Inspection of Table III, however, does not reveal any specific reason why myristic acid (S12) should be particularly important. The C18 acid was present in larger amounts and subjected to substantial variation as were the C16 and C18 saturated acids.

Keys et al.35 have concluded that stearic acid, the C18 saturated acid, has little if any effect upon serum cholesterol. Our data confirm this finding. Changes in dietary stearic acid have a very low correlation with changes in serum cholesterol (R = 0.245) and inclusion of stearic acid in the regression equation with the four important variables (myristic, palmitic, polyunsaturates and dietary cholesterol) does not improve the fit. Furthermore, the equation containing only myristic and palmitic acid, rather than total saturated acids (Fig. 2 and 4), predicts the response to cocoa butter at the high level (period 4, Table III) much better than does the equation including the total saturated acids (Fig. 1). The reports of Horlick,36 Malmros37 and Erickson et al.10 as well as the data of Ahrens et al.1 are all consistent with the conclusion that stearic acid is essentially without effect on serum cholesterol. However, Keys has concluded that since stearic acid is not involved, palmitic acid must be the saturated acid of primary interest. This does not automatically follow as we have shown.

Keys et al.4 have emphasized that mono-unsaturated acids have no effect upon serum cholesterol whereas Ahrens et al.1 and Gunning et al.2 believe that total unsaturation may be the more important variable. In general, our data support the conclusion of Keys and his group; yet, we do not consider any of the data available entirely adequate to settle this point. The first trials with olive oil (periods 3 and 21. Table III) indicated that this oil was nearly as effective as safflower oil. Repetition of these groups (periods 18 and 36) gave lower results more in line with expectation. Nevertheless, it must be emphasized that as shown in the triangular plot (Fig. 5), the oils we studied, except for olive oil, most often fall in the range of 15 to 40 per cent of monounsaturates. The saturated and polyunsaturated levels were more uniformly distributed. A similar plot of the oils studied by Keys et al.3 will show the same tendency. It can be shown in fact that with either Keys' data or our own, when selected diets are studied, regression equations with significant regression coefficients for M are found. Furthermore, since the proportion of myristic, palmitic and polyunsaturated acids in an oil seems as important and perhaps more
important than the per cent of calories of these acids consumed, we would definitely not agree with the conclusion that "Oleic acid, and perhaps other natural mono-enes, is neutral in this respect and may be isocalorically exchanged for starch in the diet without affecting the cholesterol in the blood."^{28}

It is of considerable interest that the changes in serum cholesterol are explained as well or slightly better by the percentage composition of the dietary fat than by the fatty acids expressed as per cent of calories. This conclusion is limited, of course, to the range of fat intake studied which was 22 and 38 to 40 per cent of the calories. It seems likely to us that this would not be true when very low levels of fat are used. Nevertheless, the range of fat intakes we studied included the limits of practically acceptable diets in the United States. The most effective diets appear to be those in which the percentage of myristic and palmitic acid in the dietary fat is low and the percentage of polyunsaturated acids is high. Clearly it is much easier to obtain diets of this kind when the total fat intake is high. Furthermore, since a low proportion of myristic and palmitic acid is of primary importance, the inclusion of monosaturated acids, stearic and short chain acids as well as polyunsaturated acids is helpful in achieving these kinds of diets. Dietary advice to lower the total fat intake is likely to be self-defeating.

Unfortunately, analytical data on the myristic acid content of food fats is limited. It appears to be a constituent of all animal fats although the amount may vary from 1 to 12 per cent. Most of the common vegetable oils other than coconut oil contain very little.

Dietary cholesterol is obviously an important variable in determining the serum cholesterol level. All recent work appears to support this conclusion. There does not appear to be adequate quantitative information on the serum cholesterol response to different dietary levels. As shown in Figure 6, our data indicate an essential linear response to dietary cholesterol.
and an average increase of about 5 mg. per cent serum cholesterol for each additional 100 mg. of dietary cholesterol although there is one aberrant value for olive oil. This value is consistent with the regression coefficients in the equations in Figures 2, 3 and 4 and is similar in magnitude to the responses obtained by Connor et al. Since the three lines in Figure 6 are essentially parallel, there appears to be little or no interaction between the dietary oils and dietary cholesterol. Although Anderson et al. concluded that the serum cholesterol response is proportional to the square root of the dietary cholesterol, it appears more satisfactory to consider the response as proportional to the absolute intake at the levels we have investigated. We do not agree that serum cholesterol levels are “essentially independent” of cholesterol intakes at the levels which occur in usual diets.

Following Jolliffe’s suggestion, P:S ratio has been widely accepted as a useful measure of the efficacy of dietary fat in lowering serum cholesterol. The P:S ratio is not closely related to serum cholesterol response. It should be discarded even though it has the merit of simplicity.

It must be stressed that although the correlation coefficients for various equations presented here and by Keys et al. are extremely high for biological material, the error of the estimated cholesterol change of the best equations is about 10 mg. per cent. This is a mean value obtained with ten men per group. In our data the mean values from which the data are derived also have standard deviations of about 5 mg. per cent. It is clear, therefore, that equations of this kind are of little value in predicting what may happen to the serum cholesterol of an individual. From this it also follows that changes in the serum cholesterol of two or three patients are of little value in attempting to elucidate the action of various oils or their components. The cause of this rather large biologic variation is unknown but must be recognized.

It should be emphasized that the differences in the levels of serum cholesterol of various population groups cannot be adequately explained by differences in fat intake. This is particularly evident in recent reports on some of the nomadic tribes in which low levels of serum cholesterol are found even though large amounts of butterfat are consumed.
Less striking differences are seen in the comparison of brothers in Boston and Ireland where the consumption of larger amounts of animal fats in Ireland is associated with somewhat lower levels of serum cholesterol. Evidently other environmental factors are involved. It appears likely that total calorie consumption and physical activity may modify the effects of the dietary fatty acids.

SUMMARY AND CONCLUSIONS

Data are presented concerning the response of serum cholesterol in two groups of men fed a low fat diet to which a wide variety of oils were added. The total dietary fat supplied was either 22 or 38 to 40 per cent of the calories. Dietary cholesterol supplied as egg yolk was also varied. Test periods were of four weeks' duration. The same men were repeatedly tested throughout the study. The following conclusions are drawn:

1. A multiple regression equation involving only the changes in intake of myristic acid, palmitic acid, polyunsaturated fatty acids and dietary cholesterol was adequate to explain 91 per cent of the total variance in the levels of serum cholesterol. Inclusion of other variables did not significantly improve the fit.

2. Approximately 67 per cent of the total variance in the level of serum cholesterol was explained by changes in dietary myristic acid alone. This appears to be the most important of the fatty acid components affecting serum cholesterol levels.

3. Palmitic acid has significant but much lesser effects upon the level of serum cholesterol than does myristic acid. Increases in the polyunsaturated acids lower serum cholesterol. No specific effects on serum cholesterol could be detected for stearic, lauric or shorter chain saturated acids or for monounsaturated acids except that their presence in fats lowers the proportions of myristic, palmitic and polyunsaturated fatty acids.

4. The amount of dietary fat, tested between 22 and 40 per cent of the total calories, appeared to be without influence upon the level of serum cholesterol. Consideration of only the percentage composition of the dietary fat was somewhat more effective in predicting serum cholesterol response than was consideration of the amounts of fatty acids consumed. Thus, the proportions of fatty acids in the dietary fat rather than the percentage of calories they supply is thought to be of primary importance.

5. Dietary cholesterol appeared to be linearly related to the serum cholesterol. An increase of 100 mg. in dietary cholesterol provokes a rise in serum cholesterol of approximately 5 mg. per cent. This response was independent of the effects induced by dietary fat.

6. In view of these results, the most effective practical diets for lowering the serum cholesterol should be those relatively high in total fat with (a) a small proportion of myristic and palmitic acids, particularly myristic acid; (b) a high proportion of polyunsaturated acids; and (c) a small amount of dietary cholesterol.

ADDENDUM

In the discussion of this paper, we indicated the rather severe limitations of multiple regression analysis as a means of determining the specific effects of the individual variables. Presumably the effects of each fatty acid can only be determined with security by changing each fatty acid independently and keeping the rest of the diet constant. This cannot be done with natural oils.

As this paper goes to press, we have preliminary results obtained with partially synthetic triglycerides. Trimyristin and tripalmitin were added to safflower oil and incorporated into the fat by transesterification. This is necessary since tripalmitin is not well digested. One oil contains 26 per cent myristic acid and 6 per cent palmitic acid; the other 7 per cent myristic acid and 27 per cent palmitic acid. The remainder of the fatty acids are essentially the same in both oils and consist of 53 per cent linoleic, 10 per cent oleic and 2 per cent stearic acid.* These were incorporated in the basal diet described to supply a total fat intake of 38 per cent of the calories. The experiment was designed, of course, to study the specific effect of substituting myristic acid for palmitic acid.

To our surprise the preliminary results suggest that there is no substantial difference in the

* These oils were generously supplied by Dr. F. H. Mattson of the Proctor and Gamble Company.
effect of these oils on the serum cholesterol level. Further studies are underway.

It will be recognized that oils of this kind, i.e., relatively high in linoleic acid and myristic acid, do not apparently occur in nature, and these preparations may represent an unfortunate choice. There may be complex interactions between several fatty acids on serum cholesterol which are not revealed by the multiple regression analysis. However, it may be noted that we have found the effects of mixtures of safflower oil with coconut oil and with butterfat to be well described by the regression equations given in the paper. One might have to postulate that there are unknown factors or configurations associated with myristic, palmitic and linoleic acids in natural oils which somehow yield the regression equations obtained.

As we have also emphasized, studies on man are relatively inaccurate and a single test cannot be decisive. Nevertheless, should the preliminary results be confirmed some doubt will be thrown upon all theories relating serum cholesterol response to fatty acid composition. This includes not only the specific participation of myristic, palmitic and linoleic acid that we have postulated, but earlier conclusions as to the effectiveness or noneffectiveness of linoleic acid, stearic acid, monounsaturated acids, total unsaturation, etc. Regression equations are simply descriptive and the equations we have developed appear to give the best description of the results in terms of the fatty acid composition that we can obtain. This is to say that the dietary oils act as though the specific fatty acids they contain had the activity proportional to their regression coefficients. The equations do not prove that the fatty acids have these effects. The same, of course, is true of equations involving other measures of the composition of the dietary fat.

ACKNOWLEDGMENT

We are indebted to a number of people and organizations for assistance in these studies. The facilities and patients at the Danvers State Hospital were made available by Dr. Peter P. Hagopian, Superintendent, and his support made these studies possible. The fats and oils utilized were supplied by Dr. Fred H. Mattson, Miami Valley Laboratories, Procter and Gamble Company, Cincinnati, Ohio. The milk and ice cream containing the test oils were supplied through the courtesy of the Hood Milk Company, Boston, Massachusetts, with the cooperation of Dr. H. L. Wildasin and Mr. Wallace Fogg. Dr. Jane Worcester and Miss Margaret E. Drolette of the Department of Biostatistics, Harvard School of Public Health, provided valuable consultative services in developing and interpreting the data for machine computation. Laboratory assistance was supplied by Miss Rosemary Bonanno and Mrs. Anna Gallagher. Supervision of the meals and their preparation was performed by Mrs. Elaine S. Kenneally and Miss Margaret Brown.

REFERENCES
12. Connor, W. E., Hodges, R. E. and Bleiler, R. The serum lipids on men receiving high chole-
Quantitative Effects of Dietary Fat

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