Effect of Saturated and Unsaturated Fats on the Concentration of Serum Cholesterol and Experimental Atherosclerosis

By Alfred Steiner, M.D., Aristides Varsos, M.D., and Paul Samuel, M.D.

The feeding of a diet rich in unsaturated fats to rabbits produced no significant alteration of the serum cholesterol level, while the feeding of a diet rich in saturated fats produced an elevation of the serum cholesterol without the development of experimental atherosclerosis. The addition of unsaturated fats to a diet rich in cholesterol failed to prevent hypercholesterolemia and experimental atherosclerosis in rabbits.

In a previous paper it was demonstrated that the long-term administration of a diet of 50 or 75 per cent ground peanuts to rabbits induced consistent hypercholesterolemia and led to atherosclerosis in 10 of 33 animals. Ground peanuts contain 53 per cent fat, of which 19 per cent is saturated and 81 per cent unsaturated. Since this report, Kinsell, Ahrens, Beveridge, Bronte-Stewart, and Steiner have found that the feeding of vegetable fats rich in unsaturated fatty acids to man significantly lowers the serum cholesterol level. In contrast, ingestion of fats containing a high concentration of saturated fatty acids results in an elevation of the level of serum cholesterol.

Divergent opinions prevail concerning the influence of diets rich in saturated or unsaturated fats on the serum cholesterol concentration and the development of atherosclerosis in animals. Lambert et al. have found that the feeding of safflower oil to rabbits causes a twofold increase in serum cholesterol levels after 40 days without the production of atherosclerosis, while coconut oil feeding produces an eightfold increase in serum cholesterol concentration with the development of visible atherosclerosis. However, Van Handel and Zilversmit failed to produce a significant effect on the serum cholesterol level of rabbits after 4 months on a diet containing 35 per cent of either cotton seed oil, hydrogenated cotton seed oil, or corn oil. Using rats, Avigan and Steinberg reported an increase in the serum cholesterol level on a diet, containing 20 per cent coconut oil, but failed to obtain similar results after a diet of 20 per cent corn oil. Diller et al. could not confirm this result and found that varying the degree of unsaturation of corn or safflower seed oil by means of hydrogenation did not affect the plasma cholesterol level of rats.

Similarly, various studies on the effect of different fats, saturated and unsaturated, on the development of cholesterol-induced atherosclerosis in animals have not resulted in uniform conclusions. Lambert and his co-workers produced the same degree of experimental atherosclerosis in rabbits on cholesterol enriched diets to which either hydrogenated vegetable shortening or safflower seed oil was added. However, Kritchevsky et al. administered 9 per cent fat (a hydrogenated vegetable shortening or corn oil) with 3 per cent cholesterol to rabbits and obtained higher serum cholesterol levels and more severe lesions with the shortening than with the corn oil. Kim and Ivy stated that the feeding to rats of corn oil, fatty acids, or oleic acid facilitated the cholesterol absorption far better.
than palmitic acid (in 1:24 ratio of cholesterol to fat). Stamler et al.\textsuperscript{12} in a study in chicks on a 1 per cent cholesterol diet, reported that oleic acid resulted in significantly greater cholesterol levels than did cotton seed oil, but in contrast the aortic atherogenesis was less in the animals on oleic acid.

Hegsted, Gotis and Stare\textsuperscript{13} fed 65 different oils in addition to cholesterol and cholic acid to rats. Tung oil, olive oil and tristolein preparations produced the highest serum cholesterol levels, while the use of a 50 per cent mixture of coconut and safflower oils resulted in the lowest serum cholesterol concentrations. Swell and Flick\textsuperscript{14} administered 25 per cent fat (lard, oleic acid or stearic acid) with 2 per cent cholesterol to rats and concluded that the absorption of cholesterol appeared to be decreased under the effect of highly saturated fats and hence caused a lowering of the blood cholesterol levels. Diller and his associates\textsuperscript{15} using different degrees of oil saturations could not demonstrate significant differences in plasma cholesterol levels if 2 per cent cholesterol was added to the diet. The present experiment was undertaken to clarify the effect of diets rich in saturated as compared to unsaturated fatty acids on the serum cholesterol level of rabbits and to determine the influence of the saturated as compared to unsaturated fatty acids upon the development of hypercholesterolemia and atherosclerosis in cholesterol-fed rabbits.

**Methods**

One hundred and twenty chinchilla rabbits were used. One hundred and twelve were approximately 6 months of age at the onset of the study. Eight additional animals had been kept in our laboratories on stock diet, for a period of 6 to 12 months before the beginning of the experiment and were designated as old rabbits. The animals were about equally divided as to sex. They were kept indoors in individual cages. The stock diet consisted of Ralston Purina chow containing a maximum of 2 per cent crude vegetable fat. The animals were divided into 7 groups which were given the following diets: 38 animals designated as C were fed a diet containing 0.5 per cent cholesterol; 12 animals designated as SAFF 12 were given 12 per cent safflower seed oil; 12 rabbits designated as COC 12 were fed 12 per cent coconut oil; 26 animals designated as SAFF 5C were given 5 per cent safflower seed oil plus 0.5 per cent cholesterol; 12 animals designated as SAFF 12C were fed 12 per cent safflower seed oil plus 0.5 per cent cholesterol; 12 animals designated as COC 12C were given 12 per cent coconut oil plus 0.5 per cent cholesterol; the remaining 8 old rabbits designated as SAFF 5CX were given 5 per cent safflower seed oil plus 0.5 per cent cholesterol. Table 1 summarizes the various diets together with the number of animals in each group. The safflower seed oil contained 65 to 75 per cent linoleic acid and had an iodine number of 145. The coconut oil contained 2 per cent linoleic acid with an iodine number of 10. Cholesterol U.S.P. was dissolved in ether and thoroughly mixed with the Purina rabbit chow. The ether was then allowed to evaporate leaving the cholesterol in small particle size evenly dispersed through the food. The oils were added in the indicated proportions and mixed until completely adsorbed by the stock food. The animals were fed ad lib.

The rabbits were weighed and bled from the ear veins at approximately monthly intervals. Serum cholesterol content was determined by the method of Abell and associates.\textsuperscript{16} Eighty animals completed the experiment of 100 to 125 days duration. Forty animals died during the study. The relatively high death rate was probably due to an epidemic of pneumonitis. At the end of the experiment the animals were sacrificed by injection of air into the marginal ear veins. Eighty-eight animals were examined post mortem. The degree of atherosclerosis was graded on a 0-4 scale. One plus represented several small raised TABLE 1.—Summary of Diets in Various Groups

<table>
<thead>
<tr>
<th>Group designation</th>
<th>Number of animals</th>
<th>Diet</th>
<th>Animals surviving experiment</th>
<th>Animals autopsied</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>38</td>
<td>0.5% Cholesterol</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>SAFF 12</td>
<td>12</td>
<td>12% Safflower oil</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>COC 12</td>
<td>12</td>
<td>12% Coconut oil</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>SAFF 5C</td>
<td>26</td>
<td>5% Safflower oil + 0.5% Cholesterol</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>SAFF 12C</td>
<td>12</td>
<td>12% Safflower oil + 0.5% Cholesterol</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>COC 12C</td>
<td>12</td>
<td>12% Coconut oil + 0.5% Cholesterol</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>SAFF 5CX (old rabbits)</td>
<td>8</td>
<td>5% Safflower oil + 0.5% Cholesterol</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td></td>
<td>80</td>
<td>88</td>
</tr>
<tr>
<td>Group</td>
<td>Initial Serum Cholesterol Level</td>
<td>No. of Animals</td>
<td>30 Days Serum Cholesterol Level</td>
<td>No. of Animals</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>SAPF 12</td>
<td>43 ± 20</td>
<td>9</td>
<td>38</td>
<td>9</td>
</tr>
<tr>
<td>COC 12</td>
<td>54 ± 25</td>
<td>11</td>
<td>91</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>43 ± 25</td>
<td>24</td>
<td>147</td>
<td>24</td>
</tr>
<tr>
<td>SAFF 5C</td>
<td>41 ± 16</td>
<td>16</td>
<td>114</td>
<td>16</td>
</tr>
<tr>
<td>SAFF 12C</td>
<td>43 ± 8</td>
<td>11</td>
<td>619</td>
<td>11</td>
</tr>
<tr>
<td>COC 12C</td>
<td>51 ± 16</td>
<td>10</td>
<td>927</td>
<td>10</td>
</tr>
<tr>
<td>SAFF 5CX</td>
<td>41 ± 14</td>
<td>7</td>
<td>626</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 2.—Average Serum Cholesterol Levels and Standard Deviations

RESULTS
The effects of the various diets on the serum cholesterol level of each group of rabbits throughout the study are included in table 2. The serum cholesterol levels of the rabbits in group SAPF 12 (12 per cent safflower seed oil) decreased slightly during the experiment and averaged 31 mg. per cent at the end of the study. In contrast, the serum cholesterol levels of group COC 12 increased twofold to average 110 mg. per cent, a statistically significant rise. The animals in both of these groups gained weight progressively during the course of the experiment.

The serum cholesterol values for the animals of group C (0.5 per cent cholesterol) rose promptly and averaged 365 mg. per cent at the end 125 days. The addition of either 5 or 12 per cent safflower seed oil (groups SAFF 5C, SAFF 12C and SAFF 5CX) or 12 per cent coconut oil (group COC 12) to the 0.5 per cent cholesterol-enriched diet resulted in an elevation of the serum cholesterol level to approximately a 1,000 mg. per cent after 125 days, a threefold increase as compared to that which occurred in the absence of added oil. All of the animals gained weight at the expected normal rate during the study.

Autopsy Studies. The results of the post-mortem examinations are included in table 3. No gross atherosclerosis was found in either group SAPF 12 or group COC 12. In group C, 17 of the 24 animals had gross atherosclerotic lesions in the aorta. The average intensity of the aortic lesions for the group was 1.08. A significant hypercholesterolemia (over 150 mg. per cent) did not develop in 6 of the 7 animals that were free of gross aortic lesions in this group. In group SAFF 5C, 14 of 16 animals developed gross atherosclerosis with an average intensity of the lesions of 0.94. All of the 11 animals examined in group SAFF 12C had gross lesions with an average intensity of 1.45. Seven of 10 animals of group COC 12C had gross aortic atherosclerosis with an average intensity of 1.60. In group...
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SAFF 5 CX, 5 of 7 animals examined had gross atherosclerosis of the aorta with an average intensity of 1.71.

DISCUSSION

These results indicate that feeding of a fat rich in unsaturated fatty acids did not significantly change serum cholesterol levels of the rabbit over a period of 4 months. The average decrease of 12 mg. per cent that occurred in the concentration of serum cholesterol level was not statistically significant. However, the feeding of a fat rich in saturated fatty acids caused a twofold increase of serum cholesterol levels during the same period of time. In neither of the two groups was gross atherosclerosis of the aorta present. These findings confirm an earlier report indicating that a hyperlipemia in rabbits results from the feeding of a diet rich in vegetable fats but free of cholesterol. However, the extent of elevation of the serum cholesterol level in the present study was not as great as in the previous report when 50 to 75 per cent of the diet was composed of ground peanuts. The fat content of the 50 to 75 per cent ground peanut diet was approximately 30 or 40 per cent with 81 per cent of the fatty acids unsaturated and 19 per cent saturated. The results that were obtained in this study are comparable to those reported by Lambert and co-workers.

The addition of 12 per cent safflower or coconut oil added to 0.5 per cent cholesterol in the diet caused two- to threefold higher serum cholesterol level than cholesterol alone. Factors influencing absorption of cholesterol from the gastrointestinal tract have been studied by Kim and Ivy. On the basis of the present report the mechanism of production of the difference in serum cholesterol level resulting from the addition of oil could not be explained. The degree of the atherosclerosis lesions showed no significant difference between the three groups. Although coconut oil produced higher serum cholesterol levels when fed without cholesterol than safflower oil did, the safflower oil failed to demonstrate any protective effect against hypercholesterolemia and atherogenesis when added to a cholesterol containing diet.

Old animals fed 5 per cent safflower oil plus 0.5 per cent cholesterol showed a sharp initial rise in serum cholesterol as compared to young rabbits on the same regimen. Nevertheless, identical end-results were found in both groups. Although the average intensity of the atheromatous lesions was higher in the older group of rabbits, on statistical analysis no significant difference was found between the two series. Kelly, Taylor and Hasl by comparing the reaction of young and old rabbits on a diet containing 0.15 to 0.45 per cent cholesterol and 0.85 to 2.55 per cent lipid extract of spinal cord found lower average serum concentration among the young animals as compared to the old ones during the first part of their experiment. However, similarly to our findings, the end result of the two groups showed relatively closer values. Nevertheless, they stated that "young animals were more likely than old animals to have severe atheromatosis under similar conditions." This difference could not be demonstrated in the present study by using cholesterol and safflower oil.

The divergent results obtained by the feeding of saturated and unsaturated fats in various species of animals can not be explained. It should not be inferred that the effects of the various dietary fats in the rabbit are in any way pertinent to humans.

### Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Averages serum cholesterol (mg% of fat)</th>
<th>Average aorta cholesterol level (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFF 10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>COC 10</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>C 5C</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>COC 12C</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>SAFF 5CX</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

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SUMMARY

The feeding of a diet containing 12 per cent safflower oil to rabbits fed for approximately 125 days did not significantly alter the serum cholesterol levels from the pre-experimental concentrations. The substitution of coconut oil in a second group of rabbits resulted in a twofold increase in serum cholesterol at the end of the experiment. The addition of either safflower or coconut oils to a diet containing 0.5 per cent cholesterol caused a threefold higher average serum concentration than did the feeding of cholesterol alone.

Post-mortem examination of rabbits failed to reveal experimental atherosclerosis in groups fed safflower or coconut oil (without cholesterol). Sixty-three per cent of the cholesterol fed control animals had visible atheromatous lesions. If the safflower or coconut oils were added to the diet containing 0.5 per cent cholesterol, the occurrence of lesions was slightly greater than in the animals on cholesterol alone. However, this difference was not statistically significant.

In the present study, the addition of safflower oil rich in unsaturated fatty acids failed to prevent the hypercholesterolemia and experimental atherosclerosis in cholesterol-fed rabbits.

SUMMARY IN INTERLINGUA

Dietas continente 12 pro cento de oleo de carthamo, administrate durante circa 125 dies a conilios, non alterava significativamente le concentrationes sereal de cholesterol. Le substitution de oleo de coco in un secunde gruppo de conilios resultava in un duple augmento del cholesterol sereal al fin del experimento. Le addition tanto de oleo de carthamo como etiam de oleo de coco a un dieta continente 0.5 pro cento de cholesterol produceva un concentration sereal medie de cholesterol tres vices plus alte que le administration de cholesterol sin supplemento de oleo.

Examine post morte del conilios non revelava le presentia de atherosclerosis experimental in le gruppos que habeva recipite oleo de carthamo o oleo de coco sin cholesterol.

REFERENCES

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ALFRED STEINER, ARISTIDES VARSOS and PAUL SAMUEL

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