Fatty Acid Composition of Plasma Lipids of Normal, Triton-Treated and Cholesterol-Fed Rabbits

E. Evrard, Ph.D.,* J. van den Bosch, M.D.,* J. V. Joossens, M.D.,† and P. De Somer, M.D.‡

Hyperlipemia develops in cholesterol-fed rabbits accompanied after several weeks by lipid infiltration of the intima of the aorta. In contrast, the even higher plasma lipid levels obtained by repeated injections of the non-ionic surfactant Triton WR-1339 are not associated with similar vascular lesions. This finding has aroused much interest, and several authors have studied the kinetic aspects and mechanism of this type of hyperlipemia.

After injection with Triton, plasma triglycerides immediately begin to rise, and an increase of phospholipids and both free and ester cholesterol follows with a delay of several hours. In the course of sustained treatment, triglycerides amount to over half of the total plasma lipids, while phospholipid values are higher than those of cholesterol; the relative amount of free cholesterol is also unusually high. This lipid pattern is entirely different from that in the lipemia induced by cholesterol feeding, in which half of the lipid material is present as esterified cholesterol, and the phospholipid:cholesterol ratio is reversed. This reversed ratio has been suggested to be a major cause of the atherogenic effect of cholesterol feeding.

Both types of lipemia are also different as far as the mechanism by which they are instituted is concerned. The cholesterol appearing in the plasma during cholesterol feeding is of exogenous origin, and its increase is associated with a decrease in liver cholesterol synthesis and an increase in liver cholesterol content. Triton on the other hand accelerates the endogenous production of cholesterol in the liver, from which it is continuously discharged into the bloodstream, so that the amount of liver cholesterol remains constant.

A similar mechanism to account for the rise of plasma phospholipid and triglyceride levels has not yet been described. The fact that Triton has a blocking effect on the post-heparin lipoprotein lipase and that it retards the disappearance of intravenously administered fat emulsions may at least partially explain the maintenance of high plasma triglyceride levels, although these mechanisms do not explain the rapid appearance of triglycerides in the blood stream after injection of fasted animals.

The purpose of our experiments was to investigate the fatty acid patterns of the phospholipids, cholesteryl esters, triglycerides and nonesterified fatty acids of both types of hyperlipemia. We were not only interested in the action of Triton and dietary cholesterol on the distribution and the transport mechanism of lipids, but also in a possible more profound effect at the site of synthesis and esterification of fatty acids. In this study we investigated by means of gas liquid chromatography whether there was any appearance of abnormal types or other deviations from the normal distribution of the fatty acids in the plasma fractions of the two groups of rabbits.

From the University of Louvain, Rega Institute for Medical Research and the Laboratorium voor Pathologische Biochemie, Louvain, Belgium.

* Research Assistant, Rega Institute for Medical Research; † Director, Laboratorium voor Pathologische Biochemie; ‡ Director, Rega Institute for Medical Research.

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Fatty Acid Composition of Plasma Lipids

Mathews

total lipids and the separated plasma lipid fractions of normal, cholesterol-fed and Triton-treated rabbits.

METHODS

Twenty-four normal adult rabbits were divided into three equal groups. One group was fed a diet consisting of 96 per cent commercial chow, 3 per cent groundnut oil and 1 per cent cholesterol. The composition of the commercial feed was as follows: water content 12.5 per cent; crude protein, 22 per cent; crude fiber, 4.8 per cent; mineral ashes, 7 per cent; nitrogen-free extract, 50.5 per cent; starch content (method of Ewers) 33 per cent; crude ether-soluble fat (including 3 per cent added groundnut oil), 6.5 per cent. Its fatty acid composition was: lauric acid, 10 per cent; myristic acid, 3.2 per cent; palmitic acid, 15 per cent; stearic acid, 1 per cent; oleic acid, 38.3 per cent; linoleic acid, 31 per cent; linolenic acid, 1.4 per cent; traces of palmitoleic, arachidic and behenic acids.

The same diet, without addition of cholesterol, was fed to the second group of animals which twice a week received an intravenous injection of 400 mg. per kg. body weight of Triton WR 1339, a dose which was reduced to 200 mg. per kg. in the fifth week.

The third group served as a control and had the same diet as the second group, but received no injections.

All animals gained weight and appeared healthy throughout the experiment.

Heparinized blood samples were taken by ear bleeding after zero, two, five, eight and eleven weeks. The animals were then killed and autopsy was performed to estimate the severity of their aortic lesions.

Aliquots of fresh plasma were extracted as suggested by Delsal,13 by using the cold mixture methylal: methanol 4:1. Portions of 20 ml. of extraction mixture were acidified with 2 drops of normal sulfuric acid, to insure quantitative recovery of the nonesterified fatty acids. A few crystals of hydroquinone were also added as antioxidant, as recommended by Böttcher.13

The extracts were evaporated at room temperature in vacuo until a small volume of aqueous emulsion remained, and the lipids were purified by partition between an aqueous phase and heptane with a modification of a method suggested by Faure,14 using 1.5 molar magnesium chloride instead of sodium chloride. The pH of the saline phase was found to be about 2.0. The upper heptane phase was siphoned off and the aqueous layer re-extracted three times with fresh heptane. The heptane extracts were pooled and brought to a known dilution. Aliquots were used for the following determinations: lipid phosphorus after Fiske and Subbarow,15 total ester by the hydroxamic acid method of Morgan.16 This method was preferred as an estimation of total lipids, instead of the nonspecific oxidimetric methods. It was found, however, that the formation of hydroxamic acids failed to work correctly with the extracts from cholesterol-fed rabbits, when esterified cholesterol was the major component. For these samples the dichromate colorimetric method of Bragdon17 was applied. Nonesterified fatty acids were isolated by alkaline extraction of the aliquots by 0.05 normal sodium hydroxide in 50 degrees ethyl alcohol, recovered in petroleum ether after acidification of the washed alkaline extracts, and titrated using the method of Dole.18 Previous removal of the bulk of the phospholipids by acetone-precipitation of the original petroleum ether extracts of the plasma of Triton-treated animals was found necessary. After titration, the free fatty acids were methylated for chromatography with dry methanol and paratoluene sulfonic acid as catalyst.

Cholesterol was determined following the method of Zlatkis et al.19 on separate alcohol: ether extracts, as the presence of hydroquinone interferes with the ferric chloride reagent.

Chromatography was performed with an aliquot of the total lipid extract on a 6 by 40 mm. column of silicic acid, with column loads not exceeding 5 mg., following the simplified elution scheme of Hirsch and Ahrens,20 using the sequence: 1 per cent ethyl ether in light petroleum ether; anhydrous ether and methanol. Column efficiency was tested by observing the migration of a fluorescent band corresponding to the peak of the cholesteryl esters during the elution with the first solvent. Since contamination of the cholesteryl esters by glycerides was observed during the fractionation of samples of the animals treated with Triton, chromatography was performed again on the first fraction on a second fresh column. A loss of approximately 15 per cent of the cholesteryl esters with a chain length of 20 carbons was found to occur during each chromatographic procedure, but the esters with 18 carbon atoms were quantitatively recovered.

The three eluates from the silicic acid column containing, respectively, the cholesteryl esters, a mixture of the glycerides and free cholesterol, and the phospholipids, were separately evaporated to

* Merck, Darmstadt, W. Germany.
TABLE I
Lipid Fractions of Plasma from Control and Experimental Rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control Animals (av.)</th>
<th>Triton-Treated Animals (av.)</th>
<th>Cholesterol-Fed Animals (av.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Over-all</td>
<td>Week of Treatment</td>
<td></td>
</tr>
<tr>
<td>Total ester (as triolein, mg. per 100 ml.)</td>
<td>125</td>
<td>2</td>
<td>6,180</td>
</tr>
<tr>
<td>Total cholesterol (mg. per 100 ml.)</td>
<td>62</td>
<td>5</td>
<td>1,270</td>
</tr>
<tr>
<td>Free cholesterol (mg. per 100 ml.)</td>
<td>20</td>
<td>8</td>
<td>885</td>
</tr>
<tr>
<td>Phospholipids (lecithin, mg. per 100 ml.)</td>
<td>80</td>
<td>11</td>
<td>1,330</td>
</tr>
<tr>
<td>Ratio free:total cholesterol</td>
<td>0.31</td>
<td>2</td>
<td>0.74</td>
</tr>
<tr>
<td>Ratio total cholesterol:phospholipids</td>
<td>0.78</td>
<td>5</td>
<td>0.90</td>
</tr>
</tbody>
</table>

**Note:** N. D. = not determined.
* Based on the oxidimetric determination of total lipids (see Methods).

Dryness under reduced pressure, and transformed into methyl esters of the fatty acids by the interesterification procedure and method of purification described by Stoffel et al. 21

Chromatography was performed on the methyl esters thus obtained, and the methyl esters from the nonesterified fatty acids, with an Argon gas chromatograph (Pye), with Reoplex 400 as stationary phase at 175°C. 22

Several plasma samples of each group of rabbits were also extracted and chromatography was performed after interesterification without fractionation of the lipid classes.

**RESULTS**

**Lipid Levels**

Table 1 summarizes the mean values of the major lipid classes at different moments of the eleven week treatment with Triton or cholesterol feeding. The plasma lipid response to the experimental treatment is in general agreement with the results of others.2, 7 As expected, the free:total cholesterol ratios remained about normal in the cholesterol-fed group (average 0.21), while in the Triton-treated group this value averaged 0.74. The relative rise of free cholesterol was always in great excess over the rise of esterified cholesterol. The cholesterol:phospholipid ratios were: 0.78 in the control group; 0.87 in the Triton group and 2.9 in the cholesterol group.

The determination of the nonesterified fatty acids in the presence of extremely large amounts of phospholipids was difficult to carry out, as the latter promoted the formation of undesirable emulsions. Originally this procedure yielded values of about five times those of the controls. As will be described, it could be shown that this increase was due to an artificial saponification of phospholipids during the alkaline extraction of the total lipid extract. When phospholipids were removed by acetone precipitation, previous to the alkaline extraction, normal concentrations of nonesterified fatty acids were found. Although only a limited number of dependable results could be obtained, our experiments seem to indicate that the nonesterified fatty acid levels of both Triton- and cholesterol-treated groups did not significantly differ from those obtained in the control group (about 0.5 mEq. per L.), even when the other lipid classes reached values of more than thirty times those in the control group.

**Fatty Acid Patterns**

*Plasma Total Fatty Acids.* Qualitative changes of the fatty acids induced either by
TABLE II

Fatty Acid Composition of the Total Plasma Lipids from Control and Experimental Rabbits at the Eleventh Week of the Experiment*

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Relative Retention Time (Methyl Stearate = 1)†</th>
<th>Control Animals</th>
<th>Triton-Treated Animals</th>
<th>Cholesterol-Fed Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristic‡</td>
<td>n 14:0</td>
<td>0.26</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>n 15:0</td>
<td>0.31</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Palmitic</td>
<td>n 16:0</td>
<td>0.56</td>
<td>29.5</td>
<td>27.6</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>n 16:1</td>
<td>0.55</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>n 17:0</td>
<td>0.71</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Stearic</td>
<td>n 18:0</td>
<td>1.00</td>
<td>7.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Oleic</td>
<td>n 18:1</td>
<td>1.11</td>
<td>31.6</td>
<td>36.2</td>
</tr>
<tr>
<td>Linoleic</td>
<td>n 18:2</td>
<td>1.83</td>
<td>22.6</td>
<td>25.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.57</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>n 20:4</td>
<td>1.99</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.90</td>
<td>0.65</td>
<td>0.65</td>
</tr>
<tr>
<td>Total ester (as triolein, mg. per 100 ml.)</td>
<td></td>
<td>200</td>
<td>7,130</td>
<td>1,810†</td>
</tr>
<tr>
<td>Total cholesterol (mg. per 100 ml.)</td>
<td></td>
<td>46</td>
<td>2,200</td>
<td>710</td>
</tr>
<tr>
<td>Phospholipids (as lecithin, mg. per 100 ml.)</td>
<td></td>
<td>65</td>
<td>2,340</td>
<td>390</td>
</tr>
<tr>
<td>No. of determinations</td>
<td></td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

* Fatty acid composition expressed as per cent of methyl esters, detectable on the gas-liquid chromatogram, using a moderate detector sensitivity (major peak with 1.0 to 1.5 full scale amplitude of the recorder).
† Chromatography on polyester column (Reoplex 400, at 175°C).
‡ Partially lost during the microsublimation procedure.
§ Based on the oxidimetric determination of total lipids (see Methods).

Fatty acid composition expressed as per cent of methyl esters, detectable on the gas-liquid chromatogram, using a moderate detector sensitivity (major peak with 1.0 to 1.5 full scale amplitude of the recorder).

Chromatography on polyester column (Reoplex 400, at 175°C).

Based on the oxidimetric determination of total lipids (see Methods).

Chromatography (see Methods). These restrictions do not affect the reliability of the peaks recorded from palmitic to arachidonic acids.

In this range, the number and chromatographic localization of fatty acids is identical for each type of lipemia. The absence of any abnormal peak indicates that neither cholesterol feeding nor treatment with Triton disturbs the synthesis of fatty acids in such a way that abnormal types are produced even after a relatively long period of hyperlipemia, and the essential effect of both forms of therapy seems to consist in an abnormal accumulation of common fatty acids. The data in Table II show quantitative differences between the fatty acids of each lipemia. These must be interpreted as the result of the predominance of cholesterol feeding or treatment with Triton.
TABLE III
Fatty Acid Patterns of Plasma Fractions from Control and Experimental Rabbits
(Mean Values ± Standard Deviation)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Phospholipids</th>
<th>Triglycerides</th>
<th>Cholesteryl Esters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Animals (8)</td>
<td>Triton-Treated Animals (13)</td>
<td>Control Animals (8)</td>
</tr>
<tr>
<td>Palmitic</td>
<td>±0.75</td>
<td>±4.65</td>
<td>±6.32</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>±0.74</td>
<td>±0.26</td>
<td>±1.34</td>
</tr>
<tr>
<td>Stearic</td>
<td>±4.95</td>
<td>±1.78</td>
<td>±4.37</td>
</tr>
<tr>
<td>Oleic</td>
<td>±4.42</td>
<td>±2.53</td>
<td>±6.33</td>
</tr>
<tr>
<td>Linoleic</td>
<td>±2.80</td>
<td>±3.54</td>
<td>±8.53</td>
</tr>
<tr>
<td>Linolenic</td>
<td>±0.32</td>
<td>±0.21</td>
<td>±0.67</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>±0.11</td>
<td>±0.26</td>
<td>±2.58</td>
</tr>
<tr>
<td>Ratio linoleic:oleic</td>
<td>±2.13</td>
<td>±0.10</td>
<td>±0.50</td>
</tr>
<tr>
<td>Ratio linoleic:stearic</td>
<td>±0.28</td>
<td>±0.13</td>
<td>±0.46</td>
</tr>
</tbody>
</table>

Note: Figures in parentheses represent number of determinations. N. D. = not determined.

The average results are listed in Table III. The total plasma lipid levels exceeded 1,500 mg.
per 100 ml. in the cholesterol-fed group, 4,000 mg. per 100 ml. in the Triton-treated animals.
Several remarkable differences in the distribution of fatty acids between the phospho-
lipids, cholesteryl esters and triglycerides have to be mentioned. The data in Table III were
statistically analyzed and the results of the test are given in Table IV.

(1) Phospholipids: The unsaturated fatty acids of the Triton-treated group show a pre-
dominance of linoleic acid at the expense of oleic acid, so that the ratio linoleic:oleic ratio
is increased to 2.1 against 1.2 in the control group, and 1.00 in the cholesterol-fed group.

The sum of the saturated acids equals about 47 per cent of the acids in each group, which
seems to indicate that the normal balance of

of a different lipid species in each case, as will appear from the results of the detailed analyses
of the fatty acid patterns of the major lipid classes.

Fatty Acid Patterns of Separate Lipid Fractions. It can be concluded from Table I that
two weeks of treatment with either dietary cholesterol or injections of Triton is sufficient to
produce the characteristic increase of plasma lipid levels in the animals. Prolonged treat-
ment accentuates the specific lipid composition in each group, but the drastic alterations which
occurred in the first two weeks are not profoundly affected.

Gas-liquid chromatographic analyses of the separated lipid classes were performed on
plasma samples of each group, taken at random at different moments of the experiment.
It appeared that with exception of a steady increase of oleic acid in the cholesteryl esters of
the Triton-treated rabbits, no evolution of fatty acid patterns occurred between the
second and eleventh week of treatment.
glycerol phospholipids and sphingolipids is not profoundly disturbed in the hyperlipemic status. Other investigators\textsuperscript{23,24} have observed a similar constant value of about 50 per cent of saturated fatty acids in plasma phospholipids, and these results are consistent with the concept that the typical phospholipid molecule contains one saturated and one unsaturated fatty acid.

(2) Triglycerides: After injection of Triton the fatty acid pattern of this fraction is characterized by a low stearic, high linoleic acid content. In the cholesterol-fed animals on the other hand there was a high stearic, low linoleic acid level. The other fatty acids remained almost unchanged.

It seemed interesting to investigate whether the differences in the fatty acids of the plasma triglycerides would be reflected by similar differences in the body fat. The perirenal fat capsule was removed from one rabbit of each group at the end of the experiment. For our purpose this fat may be considered as pure triglyceride, and the chromatography of its fatty acids was performed with the methyl esters obtained from the methanalysis of the total petroleum ether-soluble extract. Chromatography was performed simultaneously on the plasma triglycerides of the corresponding animals and the results are compiled in Table V. The three samples of body fat and the plasma triglycerides of the Triton-treated rabbit yielded nearly identical chromatograms. In the control rabbit the plasma linoleic acid

\begin{table}
\centering
\caption{Comparison Between the Distribution of the Fatty Acids in Plasma Total Triglycerides and in Body Fat (Perirenal Capsule) of Control and Experimental Rabbits after Eleven Weeks of Treatment}
\begin{tabular}{l|cc|cc|cc|cc}
\hline
Fatty Acid & \multicolumn{2}{c}{Control Animals} & \multicolumn{2}{c}{Triton-Treated Animals} & \multicolumn{2}{c}{Cholesterol-Fed Animals} \\
 & Body Fat & Plasma & Body Fat & Plasma & Body Fat & Plasma \\
\hline
Palmitic & 26.3 & 34.0 & 26.7 & 31.3 & 32.5 & 36.7 \\
Palmitoleic & 1.7 & 2.0 & 1.9 & 2.0 & 1.9 & 3.3 \\
Stearic & 6.2 & 7.0 & 4.4 & 4.0 & 5.4 & 14.4 \\
Oleic & 41.0 & 42.5 & 38.2 & 39.0 & 36.5 & 36.5 \\
Linoleic & 21.6 & 12.5 & 23.2 & 22.5 & 18.6 & 8.2 \\
Linolenic & Trace & 0.6 & Trace & 0.8 & Trace & Trace \\
Arachidonic & 0.0 & 0.0 & 0.0 & 0.0 & 0.0 & 0.0 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Statistical Analysis of the Fatty Acid Patterns in Table III.}
\begin{tabular}{l|c|c|c|c|c|c|c|c|c|c|c}
\hline
Fatty Acid & Shorthand Designation & \multicolumn{2}{c}{Phospholipids} & \multicolumn{2}{c}{Triglycerides} & \multicolumn{2}{c}{Cholesteryl esters} \\
& & Control versus Triton & Control versus Cholesterol & Triton versus Cholesterol & Triton versus Cholesterol & Control versus Triton & Control versus Cholesterol & Triton versus Cholesterol \\
\hline
Palmitic & 16:0 & >0.05 & >0.05 & >0.5 & >0.05 & >0.05 & >0.05 & >0.05 & >0.05 & >0.05 & >0.05 \\
Stearic & 18:0 & <0.05 & <0.05 & >0.05 & <0.05 & <0.05 & <0.05 & <0.05 & <0.05 & <0.05 & <0.05 \\
Oleic & 18:1 & >0.05 & >0.05 & <0.001 & >0.05 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 \\
Linoleic & 18:2 & <0.001 & >0.05 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 \\
Ratio linoleic:oleic & 18:1 & <0.001 & >0.05 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 \\
Ratio linoleic:stearic & 18:0 & <0.001 & >0.05 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 & <0.001 \\
\hline
\end{tabular}
\end{table}
level was low as compared to the body fat, and this discrepancy was even more pronounced in the cholesterol-fed rabbit, in which both linoleic and stearic acid levels of the circulating and depot triglycerides showed definitely different values.

The similarity of body and plasma triglycerides in the Triton-treated rabbit suggests an intensive contact between both triglyceride pools, and seems to indicate either that the increased plasma triglycerides are directly derived from the body fat itself, or that both have a common origin. In the cholesterol-fed rabbit, the difference between body and plasma triglycerides suggests a less definite familiarity of both pools.

(3) Cholesteryl esters: The plasma cholesteryl esters contain over 70% per cent of unsaturated fatty acids in each group, but in the hyperlipemic samples, the ratio of linoleic: oleic acid is clearly lowered as compared with the controls. The relative decrease of cholesteryl linoleate in cholesterol-induced hyperlipemia has been previously described, and it is especially noteworthy that the same lowering, although less marked, occurs in the endogenously derived cholesteryl esters of the Triton-induced hyperlipemia. The linoleic: stearic acid ratio is, respectively, 13.1, 8.1 and 5.8 in the control, Triton-treated and cholesterol-fed groups. There is also an reversed relationship between palmitoleic and arachidonic acids.

It must be emphasized, however, that the evolution of the cholesteryl ester patterns toward the predominance of oleate is slower in the Triton-induced hyperlipemia (Fig. 1). As already mentioned, there is a steady decrease in the linoleic:oleic acid ratio until the eighth week of administration of the detergent, whereas in the cholesterol-fed rabbits, the maximal alteration of this ratio was attained by the second week. Figure 1 shows that this different evolution of the fatty acid patterns is not related to the plasma levels of the cholesteryl esters, as these do not display similar differences of evolution.

(4) Nonesterified fatty acids: The patterns of this fraction are largely different in each experimental group. This variability must be interpreted with caution, as the values cover only a limited number of analyses. It must also be considered that even a small artefactual hydrolysis of other lipid components during analytical procedures might easily falsify the fatty acid spectrum, in view of the small absolute amount of nonesterified fatty acids normally present in the plasma.

As already mentioned, a considerable increase of free fatty acids was often measured when the alkaline extraction was performed in the presence of large amounts of phospholipids in the plasma samples of animals treated with Triton. The gas-liquid chromatography of such material invariably yielded the fatty acid pattern of the phospholipids. When the same extraction was repeated after removal of the phospholipids, normal amounts of nonesterified fatty acids were found, with a fatty acid spectrum different from that of the phospholipids. The available methods of isolation of the nonesterified fatty acids and the limited number of determinations do not provide sufficient grounds to attribute the variability of the

Fig. 1. Plasma cholesteryl esters of experimental rabbits. Change in per cent oleic and linoleic acids in the course of eleven weeks of sustained treatment with dietary cholesterol or injections of Triton.
fatty acid patterns to the exclusive influence of the experimental treatment of the animals.

COMMENTS

The fatty acid composition of the different lipid fractions of the normal lipemia shows that palmitic, stearic, oleic and linoleic acids account for the bulk of the fatty acids, while the twelve remaining peaks with a retention time not over that of arachidonic acid represent less than 10 per cent of the components. A similar distribution is found in the hyperlipemic status induced by cholesterol feeding or administration of Triton, and no uncommon fatty acids could be detected.

The saturated:unsaturated fatty acids ratio of the cholesteryl esters and phospholipids remains remarkably constant during therapy. In these fractions the only substantial alterations are confined to oleic and linoleic acids which seems freely interchangeable. The triglycerides do not show a similar stability in the cholesterol-fed rabbits, as the decrease of linoleic acid is compensated by an increase of stearic acid, which markedly enhances the saturated fatty acid content of this fraction.

The fatty acid patterns of the total plasma lipids (Table II) show that in the cholesterol-fed rabbits a relative increase of oleic and a relative decrease of palmitic acid has occurred. This can be explained by the fact that this type of hyperlipemia contains about 50 per cent of cholesteryl esters, so that the particular fatty acid spectrum of this fraction (Table III) inevitably influences the percentages found in the total lipids.

The percentages of the fatty acids of the total lipids can be calculated from the figures of the separated fractions. It will be seen that the 21.4 per cent of linoleic acid found in the total lipids of the cholesterol-fed rabbits is more than could be expected from this calculation, since the predominant cholesteryl esters contain only 15.6 per cent and the triglycerides only 11.3 per cent of this fatty acid. It must be concluded that during the separation of the lipid classes approximately 25 per cent of the linoleic acid had been lost. Since the same analytic procedure was applied to all samples of each group, it is hardly acceptable that a selective destruction of linoleic acid would have been confined to the hyperlipemic samples of the cholesterol-fed group only. The possibility remains that during the separation of the total extract on silicic acid column a lipid fraction particularly rich in linoleic acid fails to be eluted by methanol. Since in Triton-treated rabbits the amount of linoleic acid recovered in the fractions matches well with the values in the total lipid extract, the noneluted fraction would be specific for the cholesterol-fed rabbits.

The significant relative decrease of linoleic acid in the plasma cholesteryl esters of both Triton- and cholesteryl-treated animals deserves some comment. It must be borne in mind that the absolute amount of circulating linoleic acid is much higher in the hyperlipemic rabbits than in the control animals. Linoleic acid was abundantly present in the diet of the animals, and normal amounts of it were found in their body fat. This excludes any real depletion of this fatty acid, but rather suggests that the relative decrease in the cholesteryl esters must be caused by some specific metabolic alteration. A preferential utilization of cholesteryl linoleate cannot be the single reason for its relative decrease, since this would have resulted in a parallel increase of all other cholesteryl esters. The observation that linoleic acid is almost exclusively replaced by oleic acid seems to indicate that this occurs at the site of cholesteryl esterification.

One of the purposes of this study was to investigate whether the fatty acid patterns of the definitely atherogenic hyperlipemia induced by cholesterol feeding would be clearly distinguishable from the slightly atherogenic Triton-induced hyperlipemia. It appears that the high degree of saturation of the triglycerides of the cholesterol-induced lipemia is the sole deviation which is not found in the Triton-treated rabbits. In view of the heterogeneous origin of plasma triglycerides, the exact significance of this deviation cannot be derived from our results, since no separation of chylomicron and lipoprotein triglycerides was attempted. It is probable that each of these triglyceride pools is of different metabolic importance and possesses specific fatty acid
patterns. The observed differences might be due to the disproportionate increase of the triglyceride subclasses in cholesterol and Triton-induced hyperlipemias, which could be the result of a greater mobilization of triglycerides from the fat depots after injection of Triton on the one hand, and a greater proportion of exogenously derived triglycerides during cholesterol feeding on the other hand.

In man a low ratio of linoleic:oleic acid has been found in the cholesteryl esters of S, $10^9$ lipoproteins and has been connected with the atherogenic properties of the latter. In our cholesterol-fed rabbits this ratio is low for the total of plasma cholesteryl esters, and it seems not excluded that the cholesteryl esters found in our rabbits are components of similar low density lipoproteins. It will also be noted that in the Triton-treated rabbits the linoleic:oleic acid ratio is lower, although the change takes a longer time to develop (Fig. 1). This may be interpreted as an indication either of a tendency to atherosclerosis in Triton-treated animals, or that the discussed ratio is not closely related with vascular lesions. Since we did not attempt to separate the plasma lipids into different lipoprotein classes in order to study their fatty acid patterns, it is impossible to derive definite conclusions in this regard.

The absolute amounts of circulating cholesterol oleate has been reported by Gottenbos et al. to be the most suitable diagnostic criterion for dietary induced rabbit atheromatosis. As can be calculated from the data of Tables I and III, our experiments do not confirm such correlation for the slight atheromatosis due to Triton hyperlipemia.

**SUMMARY**

The fatty acid composition of plasma phospholipids, triglycerides, cholesteryl esters, non-esterified fatty acids and total lipids of cholesterol-fed, Triton-treated and normal rabbits were determined by gas-liquid chromatography. The hyperlipemia induced by injections of Triton is characterized by an increased amount of linoleic acid in the phospholipids; a triglyceride fatty acid spectrum resembling closely that of a depot fat by a high linoleic and low stearic acid level, and cholesteryl esters with low linoleic and high oleic acid content. The hyperlipemia induced by cholesterol feeding showed almost normal fatty acid patterns in the phospholipids, triglycerides with high stearic and low linoleic acid content and cholesteryl esters showing a fast and lasting alteration toward a low linoleic and high oleic acid content. The possible relation between specific plasma fatty acid alterations and incidence of atheromatous lesions in rabbits is discussed.

**REFERENCES**

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