The Extractability of Serum Lipids in Normal Subjects, Coronary Disease, Hyperlipemia, and Hypercholesteremia

By D. S. Amatuzio, M.D., Francisco Grande, M.D., and Shohachi Wada, Ph.D.

In Atherosclerosis and the diseases that favor its development there is an associated disturbance of plasma lipids. The lipids of the plasma and of the atherosclerotic plaques have been shown to be similar in composition, suggesting that the lipids of the plaques may arise from the plasma. Labeled cholesterol studies have shown that the plasma lipid is incorporated into the atherosclerotic plaque. Since cholesterol and other lipids are transported in the plasma as lipoprotein complexes, the stability of the plasma lipoprotein may be of importance with respect to the development of atherosclerosis. A study of lipoprotein stability was undertaken to find out if differences exist between normal subjects and individuals with coronary artery disease, essential hyperlipemia, and hypercholesteremia.

Subjects and Methods

Four groups of male subjects were used in this work. The group of normal individuals consisted of 30 clinically healthy men between 25 and 70 years of age. The subjects, included in the group of coronary patients, were 30 men who had a known myocardial infarction at least 6 months prior to the study. Their ages ranged between 30 and 74 years. There were also 13 subjects with known essential hyperlipemia whose ages ranged between 18 and 65 years. The last group consisted of 22 subjects with hypercholesteremia (serum total cholesterol above 275 mg. per 100 ml.) who had no other associated diseases, and of age 30 to 66 years.

All subjects led a normal life at the time of the study and they were neither on special diets nor receiving any medication. The lipid extractability from the plasma was measured by a method based on the work of Machekoert and Sandor, where fasting serum is incubated at 4 C. for 48 hours with ethyl ether containing ethanol in various concentrations.

Four different extractants were prepared to contain 2, 4, 6, and 10 per cent of alcohol by volume in peroxide-free anhydrous ethyl ether. Serum samples were obtained from 30 ml. of fasting blood by centrifugation at 2,000 rpm for 15 minutes. To each of four, 40-ml ground-glass stoppered conical tubes containing 1 ml. of serum was added 1 ml. from one of the ethanol-ether mixtures. The tubes were gently rotated for exactly 20 seconds and immediately incubated at 4 C. for 48 hours. After this period 10 ml. of petroleum ether (B.P. 39 to 56 C.) were added to each tube followed by shaking for 1 minute. After standing for 15 minutes the petroleum ether was removed by a capillary pipette, with care not to remove any of the serum layer. The petroleum ether extraction was repeated two more times, using 5 ml. of petroleum ether each time. Two series of serum aliquots were measured; one for the cholesterol determinations and another for the esterified fatty acids. Total cholesterol and the esterified fatty acids were determined using the total volume of the extracted serum by the methods of Abell et al. and Bauer and Hirsch, respectively. Cholesterol and esterified fatty acids were also estimated on a sample of the nonextracted serum. The amounts of extractable cholesterol and of extractable esterified fatty acids were calculated from the difference between the fasting nonextracted serum and the extracted serum at the various ethanol concentrations, and expressed as per cent of the corresponding amounts in the nonextracted serum.

Results

The serum of 10 individuals was analyzed in duplicate to check the reproducibility of the extraction method. The results showed a good agreement of the cholesterol release between the two sets of analysis (table 1).

Individual variability of lipid release was

*Analytical Reagent, Mallinekrodt.

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also studied on serum samples of 10 subjects on two separate occasions at intervals of several weeks. The cholesterol extractability at various alcohol concentrations was found to be reproducible as shown in table 2.

The serum extractability values of the various groups for cholesterol and for total esterified fatty acids are presented in tables 3 and 4. In all subjects the per cent lipid extraction increased with increasing alcohol concentration. The extractability of cholesterol and of esterified fatty acids was higher in the patients with coronary artery disease and in the patients with hyperlipemia than in the normal individuals.

The differences of percentage extraction between coronary patients and normal subjects and between hyperlipemic and normal subjects were statistically significant, as shown in tables 5 and 6, with the exception of the difference between coronary patients and normal persons for esterified fatty acids with the 2 per cent alcohol solution.

The per cent extraction differences for cholesterol between normal and hypercholesteremic (noncoronary) subjects were significant for the four alcohol concentrations. The total fatty acids extraction differences were significant only at the higher alcohol concentrations.

No significant differences in per cent extraction for cholesterol or esterified fatty acids were found between coronary patients and hypercholesteremic (noncoronary) subjects with the exception of the cholesterol extraction differences at the highest alcohol concentration.

Discussion

The analytical methods currently used in the study of the serum lipoproteins13-15 have failed to record a consistent difference in physical properties between the lipoprotein complexes of normal and of vascular disease states. The present data indicate that the binding of the lipids in the lipoprotein complexes, as measured by the extraction method used, is more stable in normal individuals than in patients with either coronary artery disease, hyperlipemia, or hypercholesteremia.

### Table 1

**Reproducibility of the Cholesterol Extraction Method. (Means of Pairs of Independent Analysis Run Simultaneously on 10 Serum Samples. Cholesterol Extraction as Per Cent of Total Cholesterol)**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>15.9</td>
<td>25.5</td>
<td>35.8</td>
<td>54.7</td>
</tr>
<tr>
<td>Second</td>
<td>16.7</td>
<td>25.3</td>
<td>36.0</td>
<td>55.7</td>
</tr>
<tr>
<td>Standard error of measurement*</td>
<td>±2.03</td>
<td>±3.00</td>
<td>±5.00</td>
<td>±4.29</td>
</tr>
</tbody>
</table>

*Standard error of measurement SEM = (Σ Δ²/2N)½.

### Table 2

**Individual Variation of Cholesterol Extraction on 10 Subjects. (Means of Samples Taken from Each Subject on Two Different Occasions. Cholesterol Extraction as Per Cent of Total Cholesterol)**

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>First occasion</td>
<td>21.2</td>
<td>27.7</td>
<td>45.9</td>
<td>66.9</td>
</tr>
<tr>
<td>Second occasion</td>
<td>21.4</td>
<td>30.7</td>
<td>47.4</td>
<td>62.1</td>
</tr>
<tr>
<td>Standard error of measurement*</td>
<td>±4.96</td>
<td>±9.30</td>
<td>±12.27</td>
<td>±11.01</td>
</tr>
</tbody>
</table>

*Standard error of measurement SEM = (Σ Δ²/2N)½.

The binding of cholesterol to the serum lipoproteins has been studied in animals16 and in man.17,18 The cholesterol extractability of rat serum by alcohol ether was found to be dependent in part on the cholesterol level16 and similar results were found in man.18 The extraction of lyophilized serum with chloroform revealed a greater extractability of cholesterol in nephrotic patients than in normal individuals.17 A recent study with use of trichloroethylene revealed a greater cholesterol extractability from the serum of coronary artery disease and hypercholesteremic subjects than from that of normal subjects.19 The present study of lipid release from normal serum samples with alcohol of varying concentrations in ether is in general agreement with the early reports of Macheboeuf8-10 in that the lipid extraction increases with increasing alcohol concentration, within the ranges of concentrations used. Similar results were obtained in the different disease states studied.
The coronary artery disease subjects were studied at least 6 months after their myocardial infarction, as the instability of serum lipoproteins usually exists for 2 months after injury. It is not possible, however, to decide whether the behavior of the lipid extraction observed existed prior to the coronary event. The fact that the hypercholesteremic individuals have an extraction pattern similar to that of patients with coronary artery disease might indicate that such pattern is not necessarily a consequence of the coronary event. On the other hand, the present data indicate that the extraction pattern of the coronary patients is independent of their serum cholesterol level, as shown by the comparisons between coronary patients with high and with low serum cholesterol concentrations.

The behavior of the hyperlipemic patients observed in the present study is similar to that observed by other authors in animals.

Table 3

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. of men</th>
<th>Age, years (mean)</th>
<th>Serum total cholesterol mg./100 ml.</th>
<th>2% Alcohol</th>
<th>Per cent extraction 4% Alcohol</th>
<th>6% Alcohol</th>
<th>10% Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30</td>
<td>44.5</td>
<td>236</td>
<td>± 7.0</td>
<td>±0.9</td>
<td>±1.3</td>
<td>±1.6</td>
</tr>
<tr>
<td>Coronary (cholesterol below 275 mg./100 ml.)</td>
<td>16</td>
<td>52.3</td>
<td>248</td>
<td>±6.6</td>
<td>±3.2</td>
<td>±3.9</td>
<td>±4.1</td>
</tr>
<tr>
<td>Coronary (cholesterol above 275 mg./100 ml.)</td>
<td>14</td>
<td>53.6</td>
<td>332</td>
<td>±8.6</td>
<td>±6.0</td>
<td>±7.1</td>
<td>±7.1</td>
</tr>
<tr>
<td>Coronary (all)</td>
<td>30</td>
<td>52.9</td>
<td>288</td>
<td>±9.3</td>
<td>±3.3</td>
<td>±4.4</td>
<td>±3.9</td>
</tr>
<tr>
<td>Hypercholesteremia</td>
<td>22</td>
<td>47.7</td>
<td>371</td>
<td>±19.7</td>
<td>±2.8</td>
<td>±3.2</td>
<td>±4.4</td>
</tr>
<tr>
<td>Idiopathic hyperlipemia</td>
<td>13</td>
<td>46.8</td>
<td>366</td>
<td>±35.6</td>
<td>±6.6</td>
<td>±4.8</td>
<td>±3.4</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. of men</th>
<th>Age, years (mean)</th>
<th>Total serum esterified fatty acids mE.</th>
<th>2% Alcohol</th>
<th>Per cent extraction 4% Alcohol</th>
<th>6% Alcohol</th>
<th>10% Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>28</td>
<td>44.5</td>
<td>10.5</td>
<td>±0.60</td>
<td>±1.8</td>
<td>±1.4</td>
<td>±2.2</td>
</tr>
<tr>
<td>Coronary (cholesterol below 275 mg./100 ml.)</td>
<td>14</td>
<td>51.5</td>
<td>12.1</td>
<td>±1.54</td>
<td>±2.0</td>
<td>±3.5</td>
<td>±4.9</td>
</tr>
<tr>
<td>Coronary (cholesterol above 275 mg./100 ml.)</td>
<td>11</td>
<td>50.6</td>
<td>15.5</td>
<td>±1.35</td>
<td>±4.6</td>
<td>±5.1</td>
<td>±5.9</td>
</tr>
<tr>
<td>All coronary</td>
<td>25</td>
<td>51.1</td>
<td>13.6</td>
<td>±1.08</td>
<td>±2.4</td>
<td>±3.7</td>
<td>±3.6</td>
</tr>
<tr>
<td>Hypercholesteremia</td>
<td>20</td>
<td>49.1</td>
<td>14.8</td>
<td>±1.10</td>
<td>±2.0</td>
<td>±4.7</td>
<td>±4.1</td>
</tr>
<tr>
<td>Idiopathic hyperlipemia</td>
<td>13</td>
<td>46.8</td>
<td>45.9</td>
<td>±7.76</td>
<td>±6.0</td>
<td>±4.9</td>
<td>±3.5</td>
</tr>
</tbody>
</table>
and in man in that the greatest fraction of lipid release was observed with the lowest alcohol concentration. It indicates that the hyperlipemic patients carry the largest part of their lipid in a form that makes it easily extractable with organic solvents. It is of interest that the rate of increase of lipid release observed with increasing alcohol concentration after 2 per cent alcohol extraction in these subjects is not significantly different from that observed in the normal individual. The lipid release of the serum in various disease states by the present method of study was found to be different from that of normal subjects. This might indicate a difference of the stability of the lipoprotein complexes.

**Summary**

The lipid extraction of fasting serum samples was studied with various alcohol-ether
solutions. The release of cholesterol and esterified fatty acids from the serum lipoproteins was found to be significantly different in the various disease states studied as compared to the normal subject. The fraction of lipid release from fasting serum was not related to the total level of the cholesterol or esterified fatty acids in normal subjects, coronary artery disease, and hypercholesteremia.

Acknowledgment

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References

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