Table 2. Frequency of the appearance of β-k band in the patients with and without IHD

<table>
<thead>
<tr>
<th></th>
<th>Total No.</th>
<th>In the presence of β-k band No.</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHD group</td>
<td>48</td>
<td>15</td>
<td>31.2%a</td>
</tr>
<tr>
<td>Control group</td>
<td>102</td>
<td>18</td>
<td>17.6%a</td>
</tr>
</tbody>
</table>

*P < 0.01.

Finally, we attempted to examine the frequency of the appearance of the β-k band in the patients with IHD (Table 2). The frequency of the β-k band was not different before and after the diet therapy. This frequency was also not observed in the singularity of the various types from WHO's classification for hyperlipoproteinemia. While the frequency of the appearance of the β-k band in the control group was found to have a value of 17.6%, in the IHD group the value was 31.2%. This difference was statistically significant (*p < 0.01*).

The present study indicated that the appearance of β-k band was produced by an incomplete hydrolysis of TG in the fraction of VLDL, and the characteristics of this mechanism was a TC-rich VLDL. Additionally, measurement of this frequency may serve in researching a risk factor for ischemic heart disease or atherosclerosis.

NUTRITIONAL FACTORS AND ATHEROSCLEROSIS
A study on Cholesterol Concentration in Foods - Shellfish

Iwao Fukui, Hideto Kushiro, Kazutaka Arisue, Yoshihisa Yamaguchi, Zensuke Ogawa, Chozo Hayashi, and Yuichi Yamamura

It is said that the use of foods low in cholesterol content is essential to dietary treatment, because of the close relationship between the serum cholesterol level and the onset or progress of atherosclerosis.

It has been warned that patients with serum hypercholesterolemia should refrain from intake of foods with high-cholesterol content such as eggs, shellfish, or Cephalopoda. However, it must be pointed out and stressed at this time that such analytic findings of high contents of cholesterol were obtained by incomplete pretreatment and colorimetric reaction of low specificity for cholesterol.

In order to find the accurate cholesterol contents in foods, analytic measurement methods, colorimetric, enzymic, thin layer chromatography, gas-liquid chromatography, and gas chromatography-mass spectrometry, were applied to the shellfish.

Materials and Methods

1. Materials

Five kinds of shellfish, corb shells, bloody clams, shortneck clams, clams, and oysters, were used as samples for this experiment.
2. Extraction of Lipids

The lipids used for the analyses were extracted from 100 g samples of shucked shellfish.

3. Analyses

a) Colorimetric Method
The Liebermann-Burchardt, Killian, and orthophthalaldehyde (OPA) reactions were employed.

b) Enzymic Method
The principle of this method is to first produce the free cholesterol and fatty acids by the action of cholesterol ester hydrolase on the esterified cholesterol and to produce Δ4-cholestenone and hydrogen peroxide by the action of cholesterol oxidase on free cholesterol. The hydrogen peroxide thus quantitatively produced was assayed by the colorimetric determination of the red quinone produced by the oxidative condensation of 4-aminoantipyrine and phenol in the presence of peroxidase. This method, which is said to have high specificity, was employed for cholesterol determination in this study.

c) Thin Layer Chromatography
Thin layer chromatography was applied to the extracts previously obtained for separation of the lipids. Two methods were employed for detecting the spots: The UV lamp and spraying the mixture of sulfuric acid-acetic acid (1:1). Cholesterol was detected by heating at 90°C for 15 minutes.

d) Gas-Liquid Chromatography
Shimadzu GC-5A was used for determination.

e) Gas Chromatography-Mass Spectrometry
Shimadzu LKB 9000 GC-MS was used for identification of the sterols.

Results and Discussion

1. Quantitative Findings Obtained by Colorimetry

As is shown in Table 1, the cholesterol contents obtained per 100 g of each shellfish by the Liebermann-Burchardt reaction were 216 mg in corb shells, 160 mg in bloody clams, 115 mg in shortneck clams, 113 mg in clams, and 153 mg in oysters. By the Killian reaction, the cholesterol contents were 174 mg in corb shells, 124 mg in bloody clams, 100 mg in shortneck clams, 93 mg in clams, and 154 mg in oysters. By the OPA reaction, the cholesterol contents were 191 mg in corb shells, 114 mg in bloody clams, 112 mg in shortneck clams, 99 mg in clams, and 169 mg in oysters. As is shown in Table 1, the average values of these three reactions are given in order of the content of cholesterol per 100 g of each shellfish, i.e., corb shells with 194 mg, oysters with 159 mg, bloody clams with 136 mg, shortneck clams with 109 mg, and clams with 102 mg.

Table 1. Cholesterol content in shellfish by colorimetric methods

<table>
<thead>
<tr>
<th>Shellfish</th>
<th>Liebermann-Burchardt reaction (mg/100 g)</th>
<th>Killian reaction (mg/100 g)</th>
<th>OPA reaction (mg/100 g)</th>
<th>Colorimetric method (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Corb shell</td>
<td>216</td>
<td>174</td>
<td>191</td>
<td>194</td>
</tr>
<tr>
<td>2. Bloody clam</td>
<td>160</td>
<td>124</td>
<td>114</td>
<td>136</td>
</tr>
<tr>
<td>3. Shortneck clam</td>
<td>115</td>
<td>100</td>
<td>112</td>
<td>109</td>
</tr>
<tr>
<td>4. Clam</td>
<td>113</td>
<td>93</td>
<td>99</td>
<td>102</td>
</tr>
<tr>
<td>5. Oyster</td>
<td>153</td>
<td>154</td>
<td>169</td>
<td>159</td>
</tr>
</tbody>
</table>

Average of triple determination.