Clinical aspects of cholestanol metabolism

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Original title: Cholestanol no rinsho

From: Igaku No Ayumi 99: 1-5, 1976

Translated by the Translation Bureau (MI/PS)
Multilingual Services Division
Department of the Secretary of State of Canada

Department of Fisheries and the Environment
Fisheries and Marine Service
Halifax Laboratory
Halifax, N.S.

1977

18 pages typscript
Clinical Aspects of Cholestanol Metabolism

Cholestanol no Rinsho

Reference in English: Strides of Medicine

Publisher: no info.
Date of publication: 1976
Volume: 99
Issue No: 1
Number of typed pages: 16

Place of publication: no info.

Requesting department: Environment
Branch or division: Fisheries & Marine
Translator (initials): MT/PS

Your number: 1089344

Date of request: 16.04.77

Unedited translation
For information only
Traduction non revue
Information seulement
CLINICAL ASPECTS OF CHOLESTANOL METABOLISM

(Cholestanol no Rinsho)

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Igaku no Ayumi (Progress of Medical Sciences)

The fact that 5α-cholestan-3β-ol (cholestanol, dihydrocholesterol) exists in mammals and that its chemical structure resembles that of cholesterol was already clarified by Diels, Abderhalden, Willstaetter, Mayer and others early in this century. Schoenheimer et al demonstrated in 1930 that cholestanol always coexists with cholesterol in a quantity varying from 1 to 2% of cholesterol in normal tissues. In 1958, Kritchersky stated that although a 5 to 10 g quantity of cholestanol, calculated from the amount of cholesterol, should exist in the human body, its significance was not known.

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SEC 5-25T (6/76)
In 1937, van Bogaert et al. reported a case of nervous ailment, in which cholesterol-like substances accumulated in the patient's central nervous system, accompanied by the symptoms of xanthoma, juvenile cataracta, progressive cerebeller ataxia and dementia, and the patient died after finally showing the symptoms of pseudo bulbar. From then on, the disease had been called spinal cholesterolosis. Later in 1968, Menkes et al. discovered that the substance accumulated was cholestanol, and the disease was reported by the name, cerebrotendinous xanthomatosis (CTX).

Philippart et al. concluded that since the cholestanol level was quite high in the blood and in the tendon xanthoma of the patient, CTX was a general abnormal metabolism of cholestanol. Since then, cholestanol started to draw a wide attention, and a number of reports started to appear on the mechanism of cholestanol metabolism and symptoms of the disease. Therefore, it is no exaggeration to say that the study of cholestanol made progress as the study of CTX did.

1. Chemistry of Cholestanol

In cholestanol, three cyclohexane rings (A, B and C) are condensed together as in phenanthrene, and at the end, a cyclopropane ring (D) is combined. The B-C ring is joined to A and D rings in a trans configuration, and fixed in a chair form. The two internuclear methyl groups at carbons 10 and 13, the hydroxyl group at carbon 3, the
the hydrogen at carbon 8 and the side chain at carbon 17 are oriented in \( \beta \) configuration. Consequently, the molecule is a highly stabilized \( \text{allo} \) compound. Cholesterol is different from cholestanol only in that the \( C_5-C_6 \) linkage is a double bond. Reduction of this double bond of cholesterol yields in addition to cholestanol, coprostanol (\( \beta \)-normal), which is a major sterol in feces.

In order to describe the clinical aspects of cholestanol, bile acids, which are a marginal group of \( C_{24} \) steroids, are important. In human bile acids, four compounds have been identified. They are cholic acid, deoxycholic acid, chenodeoxycholic acid and lithocholic acid. In all these compounds, the \( \text{OH} \) group are oriented in \( \alpha \) configuration, and the \( A/B \) ring junction is \( \text{cis} \). Cholic acid is contained in the largest proportion of all the bile steroids. In the bile, these acids exist as amides of such amino acids as glycine and taurine; they are water soluble, have a strong surface activity and are subjected to bacterial conversion by intestinal microbial flora and then introduced into the intestine-liver metabolic cyclic route.

2. Measurement of Blood Cholestanol Level and Normal Value

Cholestanol exists in the body accompanied by a much larger quantity of cholesterol. As the chemical structures
are quite alike, quantitative measurement of cholestanol has been extremely difficult in the past.

Trustwell et al.\(^3\) employed a two-step thin layer chromatographic method (TLC), which included a reversed phase, to separate \(\Delta^7\)-cholestenol and cholestanol from cholesterol.

The blood level of cholestanol in normal human was probably first determined by Salen et al. (1971)\(^{17}\), who employed a combination method of preparative TLC and gas-liquid chromatography (GLC) and reported a value of 0.9 ± 0.2 mg/dl. This method, however, required three compounds, namely, \(^{14}\)C-cholesterol, \(^{3}\)H-cholestanol and cholestane, as the internal standards to check the recovery rate of the blood cholestanol. The authors\(^3\) have developed a new method by applying mass fragmentography. By the method, an assay result can be obtained quickly (15 minutes) with good reproducibility using a small amount of the blood plasma, and furthermore, cholesterol can be determined simultaneously. According to this method, the cholestanol level of normal subjects with an empty stomach was 0.34 ± 0.05 mg/dl, which was in excellent agreement with the 0.33 mg/dl value reported by Ishikawa et al.\(^{28}\) and obtained by the direct GLC method. In their GLC method, retention times of cholestanol and cholesterol were about 60 minutes, and also it was required to change the GLC detector sensitivity after observing the internal standard peak, and furthermore the cholesterol content could not be determined simultaneously.
The large content value reported by Salen et al appears to be due to insufficient separation of cholesterol by the preparative TLC. The cholestanol levels measured by the authors' method in CTX patients are compared with the values reported by Salen (Table 1).

3. Biosynthesis and Metabolism of Cholestanol

As described above, the study of cholestanol metabolism advanced as the pathological exploration of the CTX disease. The number of cases of CTX after the first case reported by van Vogaert is considered to have amounted to over thirty in the world, and in this country, it appears that there have been six cases including the first reported by Ogawa et al\(^3\)\(^0\). Presently, CTX is considered to be autosomal recessive, congenital metabolism anomaly.

As the biochemical characteristics of CTX, the following points may be cited.

(1) Even though the blood cholesterol level could be normal or below normal in most of the cases, cholestanol level is several-fold higher than the normal level.

(2) In xanthoma, the content of cholestanol ester is large, but generally speaking, the cholesterol content in the xanthoma is also large comparing to the blood level of cholesterol.

(3) Lowering of bile acid production.

These points will be discussed in more detail in
the following section, in the order of the description above.

(1) As shown in table 1, the cholestanol level was found in a range of 1.7 to 3.1 mg/dl for the three cases found by the authors, and the level is 5 to 9 times higher than normal. The mother of one of the three patients showed a level of cholestanol about 3 times higher than normal, although she did not show any nervous symptoms. On the other hand, the cholesterol level was normal or below in all cases.

Much research work has been carried out to explore the pathology of the disease. Detection of sterol from the brain can be performed even on a brain stored at -20°C for one year after the dissection for autopsy\(^\text{16}\), or on the samples stored fixed in formalin. In either case, cholestanol deposition\(^\text{16}\) on myelin is clearly shown. The deposition occurs most frequently in the cerebellum. Medwin et al\(^\text{15}\) intravenously injected 40 \(\mu\)curies of [\(4^{-14}\)C] cholesterol to a CTX patient, and found that the brain was pathologically intact after three years when the patient's body was dissected for autopsy. However, radio-activity was detected from the brain in the form of free cholesterol and cholesterol ester together with cholestanol and cholesterol. Namely, although cholestanol can exist in the CTX brain in both free and ester forms, the latter is found in a larger content, or about 50% of the total sterol esters\(^\text{10}\). When fatty acid esters of cholesterol and cholestanol were examined by North et al\(^\text{29}\), significant differences between the two esters were found. The route of \textit{in vivo} synthesis of
cholestanol was clarified by Werbin et al.\textsuperscript{2}) in as early as 1964. Namely, they fed germfree guinea pigs food containing $[4\beta-\text{H}]$ cholesterol and $[4-\text{C}]$ cholesterol, and proved that conversion of cholesterol to cholestanol took place in the tissues, based on the measurement of radioactivities of cholesterol and cholestanol in the adrenal body, the liver and the small intestine.

The main biliary metabolite of cholestanol is allocholic acid\textsuperscript{4}). The cause of the high cholestanol level could be attributed to hindrance of cholestanol degradation, but Salen and his colleagues\textsuperscript{17, 18}) worked proved that it was rather due to the exasperation of the cholestanol biosynthesis. Namely, Salen et al.\textsuperscript{20}) intravenously injected radio-active cholesterol and cholestanol in two CTX patients, confirmed that cholestanol was synthesized from cholesterol, and concluded that the abnormal deposition of cholestanol in the CTX patient is due to exasperation of the normal cholestanol biosynthesis route. Prior to this work, Salen\textsuperscript{17, 18}) showed in 1971, that exasperation of sterol synthesis was noted only in the liver and that the synthesis product was transported to the brain and xanthoma, where it deposited, by examining six CTX patients. They later studied sterol balance and isotopic kinetics on CTX patients and on eleven controls including a hyperlipemia patient, and found that the kinetics of both cholesterol and cholestanol follow a two-pool kinetic model. They also discovered that with respect to the metabolism of cholestanol, the CTX

\textsuperscript{*}Translator's note: sic in original.
patients showed a concentration of cholestanol in the blood, a
total body pool and a production rate 2-5 times higher than in the
controls and also the same degree of higher rate of cholestanol
formation than the controls. With respect to the metabo-

lism of cholesterol, although both the rate of decay and the
rate of formation of cholesterol were twice as large as those
of the controls, concentrations of cholesterol in both the
large reserve pool (blood) and the rapidly changing kinetic
pools were smaller in the CTX patients than in the controls. These findings clearly indicate that in the CTX patients,
cholesterol synthesis is exasperated and at the same time,
degradation of cholesterol is also accelerated. In con-

clusion, the cause of CTX disease is considered to be the
exasperation of synthesis of neutral sterols in the liver.

(2) Even though the cholesterol blood level of the
CTX patients is normal, the cholesterol content in xanthoma
is extremely large. It is quite understandable that the
cholesterol content in the xanthoma is large in hypercholeste-
rolemia patients, but the same phenomenon in the CTX patients
can hardly be explained. It is assumed that because of
high blood concentration, cholestanol affects the
stability of plasma lipoproteins of both cholesterol and
cholestanol. The authors' results, summarized in
table 2, on blood level determinations of lipoproteins in
the CTX patients also showed that the cholestanol/cholesterol
ratio of the patient was different from that of the controls.
It is quite reasonable to assume that there exist some diseases which also show hypercholestanolemia, even though they are not CTX. In such diseases, it is very likely that the cholestanol/cholesterol ratio of the patient is smaller than that of CTX patients.

(3) Salen\textsuperscript{17, 18, 24} performed cholestanol kinetic study and analysis of bile acid composition of CTX patients and pointed out that the bile acid biosynthesis was sluggish in the CTX patient. Tint and Salen\textsuperscript{26} demonstrated that in the CTX patients, the sterol synthesis takes place irreversibly via the course of mevalonate $\rightarrow$ squalene $\rightarrow$ lanosterol $\rightarrow$ $\Delta^7$cholestenol $\rightarrow$ cholesterol $\rightarrow$ cholestanol. Therefore, it is conceivable that the exasperation of neutral sterol synthesis in the CTX patients occurs as a result of inhibition of the bile acid synthesis. This speculation suggest that there are at least two alternative routes in the metabolism of cholesterol, in addition to the hitherto known metabolism route to steroid hormones.

In 1971, Salen\textsuperscript{17, 18} demonstrated that chenodeoxycholic acid was missing in the CTX patients.

Since bile acids are synthesized in the liver through the negative feed back route regulated by two enzymes, cholesterol $7\alpha$-hydroxylase and HMG-CoA reductase\textsuperscript{19}, another possible cause of the CTX should be taken into consideration, and that is inhibition of the mechanism of the negative feed back synthesis of bile acids.
In mammals, cholesterol is converted in the liver to primary bile acids, which are secreted into the bile and further subjected to bacterial conversion by intestinal micro-organisms to secondary bile acids and then introduced into the enterohepatic circulation. In most of the mammalian animals, the primary bile acids are dominant, but rabbits are exceptions, and the secondary bile acid, namely, deoxycholic acid is dominant.

Accordingly, Hofmann et al.\(^{13}\) examined bile acid composition of germfree rabbits and found that cholic acid was dominant at 94%, but when the germfree rabbits were returned to the normal rearing conditions, deoxycholic acid became the dominant bile acid, and they thus confirmed that this process inhibited the synthesis of cholic acid. If we assume that there also exists such a compound derived from the secondary bile acid and inhibiting the bile acid biosynthesis in humans, then a new route in exploring pathology of CTX could be opened.

Lately, Setoguchi, Salen and Mosbach\(^{25}\) found that contents of two cholesterol-derived bile alcohols, namely, 5β-cholestan-3α, 7α, 12α, 25-tetra-ol and 5β-cholestan-3α, 7α, 12α, 24α, 25-penta-ol are high in the feces and the bile of the CTX patients, by gas chromatography-mass spectrometry. They explained that these compounds were produced as a result of inhibition of the oxidation of cholesterol side chain. It is quite interesting that they emphasized the inhibition
of negative feedback regulation to form bile acids as a cause of the CTX disease.

Although not directly related to the bile acids, it may be important to take into consideration a mechanism that may exasperate cholestanol biosynthesis as a result of mutual interaction of neutral sterols, which are precursors of cholestanol, as another cause of the CTX. Importance of this possible cause is verified by the following findings. Namely, in 1966, Shefer et al\textsuperscript{16} examined metabolic pathways of neutral sterols derived by decay of cholesterol and found that the activity of cholesta-4-en-3-one 5\alpha-reductase present in the microsomal fraction of rat liver was inhibited by cholesta-5-en-3-one and stimulated by cholesta-4,6-dien-3-one.

As described above, the cause and pathological characteristics of the CTX disease are presently explained as the abnormal exasperation of the neutral sterol synthesis system as a result of blocking of the bile acid synthesis route, as seen in the absence of chenodeoxycholic acid.

However, there are still a number of questions remaining unsolved before the cause of the CTX disease is completely clarified. These are, for example, why the cholesterol level in the blood has to be maintained at a normal value when both decay and biosynthesis of cholesterol are exasperated, why the steroid hormone biosynthesis is not exasperated but the neutral sterol route only is, while cholesterol decay is exasperated.
The authors believe that the clarification of the feedback systems in the three routes of biosyntheses of bile acids, neutral sterols and steroid hormones is the important key point for the future exploration of the pathology of CTX.

4. Other Clinical Aspects of Cholestanol

1) Atherosclerosis

The aorta of CTX patients shows presence of the atheromatous plaque, in which a deposit of cholestanol is observed, and according to Stanburg\textsuperscript{22}, myocardial infarct was observed in two cases out of 12 CTX patients. Atherogenicity (Nichols, 1955) of experimental animals fed cholestanol-containing food appears to indicate that cholestanol is also related to atherosclerosis, as cholesterol is. It is quite interesting to know that according to Gilbert \textit{et al}\textsuperscript{14}, who analyzed the lipid inside the atheromatous plaque of human aorta by GLC, the content of cholestanol was larger than that of cholesterol.

2) Experimental Gallstone Disease

Hofmann \textit{et al}\textsuperscript{7} fed rabbits cholestanol food to cause experimental gallstone disease and then examined the effects of neomycin administration. By this very well designed experimental method, they showed that cholestanol administration caused only a change of the 5β/5α ratio in the bile acids,
while neomycin administration yielded only a change of the primary/secondary ratio of the bile acids. Based on these results, they concluded that the gallstones are formed as a result of the change in the compositions of bile acids rather than by the bacterial conversion of glycoallocholic acid to allodeoxycholic acid. In other words, they proved that the ordinary intestinal microbial flora could cause the gallstone formation, if cholestanol-containing food was administered.

Conclusion

It has been very well known that cholesterol is one of the essential chemical substances to compose the living membranes. Cholestanol however has a level of 1/500 of cholesterol in the blood, and is very similar to other chemical structures. Therefore it has been hidden behind cholesterol. However, since the CTX disease was first detected, the significance of presence of cholestanol is being explored. This minute quantity of cholestanol should have a meaning that is different from that of cholesterol, and therefore, clarification of the significance of the presence is an important subject in the future.

The authors are indebted to Professor K. Kosaka and professor T. Yamakawa for their guidance throughout the work.
Table 1

Cholesterol and cholestanol concentration of plasma and erythrocyte stroma.

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Erythrocyte stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cholesterol</td>
<td>cholestanol</td>
</tr>
<tr>
<td></td>
<td>mg/dl</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Normal</td>
<td>225±25</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>M.D.</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>E.D. S.</td>
<td>127</td>
<td>2.2</td>
</tr>
<tr>
<td>E.D. E.</td>
<td>117</td>
<td>2.2</td>
</tr>
<tr>
<td>J.C.</td>
<td>129</td>
<td>1.3</td>
</tr>
<tr>
<td>G.C. B.</td>
<td>144</td>
<td>1.4</td>
</tr>
<tr>
<td>V.N.</td>
<td>196</td>
<td>3.9</td>
</tr>
<tr>
<td>Seyama &amp; Ichikawa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (CTX)</td>
<td>197.6±44.5</td>
<td>0.34±0.05</td>
</tr>
<tr>
<td>A.Y.</td>
<td>178</td>
<td>1.96</td>
</tr>
<tr>
<td>K.Y.</td>
<td>179</td>
<td>1.65</td>
</tr>
<tr>
<td>S.G.</td>
<td>208</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* ratio: cholestanol/cholesterol (%)
Table 2

Cholesterol/cholesterol ratio of plasma lipoproteins

<table>
<thead>
<tr>
<th>Seyama &amp; Ichikawa</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>control</th>
<th>CTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.192 \times 10^{-2}</td>
<td>0.236</td>
<td>0.031</td>
<td>2.75 \times 10^{-2}</td>
<td>1.91</td>
<td>0.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salen</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.64</td>
<td>2.10</td>
<td>1.42</td>
<td>1.68</td>
<td>0.76</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Table 3 Dominant bile acids in the bile

<table>
<thead>
<tr>
<th>treatment</th>
<th>none</th>
<th>neomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>0/7</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>deoxycholate</td>
<td>deoxycholate</td>
</tr>
<tr>
<td></td>
<td>cholate</td>
<td>cholate</td>
</tr>
<tr>
<td>1% cholesterol</td>
<td>13/14</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>deoxycholate</td>
<td>deoxycholate</td>
</tr>
<tr>
<td></td>
<td>cholate</td>
<td>cholate</td>
</tr>
<tr>
<td></td>
<td>allodeoxycholate</td>
<td>allodeoxycholate</td>
</tr>
<tr>
<td></td>
<td>all chocholate</td>
<td>all chocholate</td>
</tr>
</tbody>
</table>

The box at the upper left corner indicate the ratio of formation of gallstone. For example, 13/14 indicates that 13 rabbits out of 14 produced gallstones.

by Hofmann et al. \(^7\)
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33) Selen, G.: Cholestanol deposition in cerebro- 


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