Abnormalities of bile acids in serum and bile from patients with myotonic muscular dystrophy

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Summary

1. Serum bile acids in seven patients with adult type myotonic dystrophy and 22 normal persons were quantitatively analysed by gas–liquid chromatography and gas chromatography–mass spectrometry for cholesterol, $\gamma$-glutamyltransferase and bilirubin. There was no bile obstruction in any patient.

2. Deoxycholic acid values in all mothers of patients with congenital type myotonic dystrophy were three times (2.1 pmol/l) that of the control (0.7 pmol/l).

3. Uncommon bile acids were detected in the patients’ sera. One of them appeared to be dihydroxymono-oxocholanic acid, having a longer side chain. Another one appeared to be dihydroxycholanic acid, with a steroid-nucleus structure similar to chenodeoxycholic acid and with a longer side chain.

4. Biliary bile acids from three patients and one normal person were also analysed, and this revealed a remarkable decrease in ursodeoxycholic acid in the patients.

5. The presence of bile acid abnormality in patients with myotonic muscular dystrophy is proposed.

Key words: bile acids, deoxycholic acid, 3β-hydroxylated bile acids, myotonic dystrophy, ursodeoxycholic acid.

Abbreviations: MMD, myotonic muscular dystrophy; RRT, retention time relative to deoxycholic acid; $\gamma$-GT, $\gamma$-glutamyltransferase; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; HCA, hyocholic acid, $\beta$-MCA, $\beta$-muricholic acid; iso-UDCA, 3β,7β-dihydroxy-5β-cholan-24-oic acid; iso-CDCA, 3β,7α-dihydroxy-5β-cholan-24-oic acid.

Introduction

Myotonic muscular dystrophy (MMD), causing general disturbances in addition to muscle dysfunction, is apparently characterized by a metabolic morbidity resulting in generalized abnormalities. The relations between MMD and cholesterol metabolism have been demonstrated in human and animal experiments with administration of an inhibitor of cholesterol synthesis [1]. Cholesterol metabolism is regulated quantitatively by bile acids. In patients with MDD gallstones are a common occurrence [2]. This suggests that a disturbance in bile acid metabolism may underlie the pathogenesis of MMD. These observations led us to analyse and identify the serum and biliary bile acids in patients with MMD.

This paper describes the unusual composition of bile acids in serum and bile from patients with MMD, and proposes some explanation of the abnormalities of bile acids in these patients.

Methods

Clinical

Seven patients (two males and five females, age range 29–55 years) were studied as out-patients...
or in the hospital. All individuals had normal diets and none was receiving medication.

The patients in the hospital were asked to fast for 12 h. Blood samples from both out- and in-patients were collected with the patients' consent and after clotting serum was separated by centrifugation. Portions of the serum were routinely examined for free and esterified cholesterol, γ-GT and total and direct bilirubin. For the quantitative analysis of bile acids, 3.5 ml of serum was used.

Control sera were obtained from seven males and 15 females, age range 21–68 years, with normal liver function, which was checked by assay of cholesterol, γ-GT, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea nitrogen, total protein and electrolytes.

The subjects were divided into three groups: 1, a group of five females with adult MMD, mean age 41 years, having an affected child (children) with early onset MMD; 2, a group of two males with adult MMD, mean age 36 years; 3, 22 normal subjects whose sera were mixed and divided into five aliquots.

Bile was also collected by duodenal intubation after injection of cholecystokinin from two women, ages 35 and 29 years, of the first group, from a 13-year-old man of the second group, and from a healthy 27-year-old woman of the third group. For the analysis of bile acids, 0.1 ml of bile was used.

**Extraction of bile acids from serum and their separation into sulphated and non-sulphated fractions (Scheme 1)**

Bile acids in serum (3.5 ml) were extracted with a column of Amberlite XAD-2 by the method of Ikawa et al. [3]. The extract was fractionated into sulphate-conjugated and non-sulphate-conjugated fractions through a column of Sephadex LH-20 (4 g) by the method of Makino et al. [4].

After the sulphated fraction was solubilized by the method of Almé et al. [5], both the sulphated and non-sulphated fractions were hydrolysed in KOH solution (2 mol/l) at 130°C for 4 h, and then β-MCA (4 μg) was added as an internal standard.

The free bile acids liberated from the sulphated fraction were extracted with 4 × 20 ml of ether

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**Scheme 1. Quantitative determination of serum bile acids**
Bile acid abnormality in myotonic dystrophy

Extraction of free bile acids from serum

Silicic acid column chromatography


Preparation of derivatives

t.l.c. → GC–MS analysis

Scraping of the zone of UDCA → Figs. 2–7

Re-extraction → Trimethylsilylation → GC–MS analysis → Figs. 8, 9

Scheme 2. Identification of serum bile acids after group separation by silicic acid column chromatography

E/C, Ethanol/chloroform (parts v/v shown in parentheses). GC–MS, Gas chromatography–mass spectrometry.

after acidification to pH 1 with HCl (6 mol/l). The free bile acids obtained from the non-sulphated fraction were extracted with 4 × 20 ml of ether under the same acidic conditions, after removal of unsaponifiable matter by extraction of the alkaline hydrolysate with 2 × 5 ml of hexane. The hexane extract was separated by centrifugation and extracted with ether in the same way.

The pooled extracts were washed with portions (5 ml) of water to neutrality and dehydrated over anhydrous sodium sulphate (10 g). The ether extract was filtered off and evaporated to dryness.

Purification of bile acids in the non-sulphated fraction by column chromatography on aluminium oxide

For further purification, free bile acids obtained from the non-sulphated fraction were methylated with diazomethane in the usual way. They were transferred quantitatively to an aluminium oxide column (2 g) with about 10 ml of benzene/hexane (1:9, v/v) and the column was washed with approximately 40 ml of the same solvent mixture. All bile acid methyl esters were then eluted with 60 ml of methanol/aceton (1:9, v/v).

Group separation of bile acids by column chromatography on silicic acid (Scheme 2)

Bile acids were chromatographed on a column of silicic acid (5 g) by a modification of the method of Ogura et al. [6], being eluted successively with portions (100 ml) of various ethanol/chloroform mixtures [2:98 (I), 4:96 (II), 6:94 (III) and 10:90 (IV), v/v]. As a result of this procedure, each fraction contained mono-hydroxycholanic acid (I), dihydroxycholanic acid (II), β-muricholic acid (III) and other tri-hydroxycholanic acids (IV) respectively.

Analysis by gas–liquid chromatography

Samples of methylated bile acids were acetylated in acetic anhydride at 140°C for 4 h. Acetyl derivatives of bile acid methyl esters were dissolved in acetone and analysed by g.l.c. under the conditions described by Ayaki [7].

Mass spectrometry

Mass spectra of bile acids were obtained on a Hitachi M-80 gas chromatograph–mass spectrometer (Hitachi Co., Tokyo, Japan). To obtain the mass spectra of individual bile acids, samples were injected into a glass-coil column
(2 m × 3 mm i.d.), packed with 2% OV-1 on 80/100 mesh Chromosorb-W (acid-washed, dimethylchlorosilane). The following operating conditions were employed; ionization voltage, 20 eV (electron impact); accelerating voltage, 3 kV (max. m/e 1500); ion source temperature, 180°C; column temperature, 230°C; carrier gas, He (40 ml/min).

**Recovery of bile acids from serum**

Recovery of serum bile acids throughout the whole analytical procedure was checked with serum samples to which various bile acids had been added.

The recovery from the stage of extraction of bile acids from serum to that of hydrolysis of conjugated bile acids (glycocholate, taurochenodeoxycholate and deoxycholate) and sulpholithocholate (after solvolysis), as measured by g.l.c., was 86·0 ± 7·2% (mean ± SD). The recovery from the stage of extraction with ether to that of analysis by g.l.c. was corrected by addition of β-MCA as an internal standard. Results from every quantitative analysis by g.l.c. were corrected for the sensitivity of the g.l.c. method.

**Detection of iso-UDCA and iso-CDCA in serum**

Bile acids in serum (20 ml from two females with adult type MMD, and serum from normal persons) were extracted with Amberlite XAD-2 and treated by alkaline hydrolysis after solvolysis. Free bile acids were extracted with ether, and chromatographed on a column of silicic acid.

**Table 1. Analysis of serum cholesterol, γ-GT and bilirubin in the control subjects and patients with myotonic muscular dystrophy (MMD)**

Each serum sample used for the group 3 normal control mixed serum was checked to verify that values for cholesterol, γ-GT, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea nitrogen, total protein and electrolytes were within the normal limits. The means (± SD) shown for group 3 were obtained routinely by the Central Laboratory. N.S., Not significant.

<table>
<thead>
<tr>
<th>Category of subjects</th>
<th>Subject no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Cholesterol (mg/dl)</th>
<th>γ-GT (i.u./l)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Free</td>
<td>Esterified (%)</td>
</tr>
<tr>
<td>Group 1 Female patients with adult type MMD having affected child of early onset type MMD</td>
<td>1</td>
<td>29</td>
<td>F</td>
<td>115</td>
<td>33</td>
<td>71-3</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>F</td>
<td>143</td>
<td>39</td>
<td>72-7</td>
<td>4-0</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>F</td>
<td>164</td>
<td>45</td>
<td>72-6</td>
<td>28-0</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>F</td>
<td>184</td>
<td>51</td>
<td>72-3</td>
<td>99-0</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>F</td>
<td>197</td>
<td>64</td>
<td>67-5</td>
<td>15-0</td>
</tr>
<tr>
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<td>(32-7)</td>
<td>(11-9)</td>
<td>(2-18)</td>
<td>(38-6)</td>
<td>(0-11)</td>
<td>(0-0)</td>
</tr>
<tr>
<td>Group 2 Male patients with adult type MMD</td>
<td>6</td>
<td>31</td>
<td>M</td>
<td>204</td>
<td>55</td>
<td>73-0</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>M</td>
<td>135</td>
<td>38</td>
<td>71-9</td>
<td>40-0</td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>(48-8)</td>
<td>(12-0)</td>
<td>(0-78)</td>
<td>(90-5)</td>
<td>(0-0)</td>
<td>(0-0)</td>
</tr>
<tr>
<td>Groups 1 and 2 (± SD) for female and male patients with adult type MMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Group 3 Mean (± SD) normal control serum (mixture from seven males and 15 females, ages 21–68 years)</td>
<td></td>
<td></td>
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<tr>
<td>Significance of differences between controls and adult type MMD patients</td>
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</tbody>
</table>
The substances eluted with ethanol/chloroform [2:98 (I) and 4:96 (II), v/v] were collected and their methyl esters were analysed by thin-layer chromatography with chloroform/acetone/methanol (110:35:3, by vol.). The zones having the same \( R_F \) as UDCA, 3\( \beta \),7\( \alpha \)-dihydroxy-5\( \beta \)-cholan-24-oic acid and 3\( \beta \),7\( \beta \)-dihydroxy-5\( \beta \)-cholan-24-oic acid, were scraped together and purified by column chromatography on silicic acid (5 g). The material eluted with 50 ml of ethanol/chloroform (4:96, v/v) was converted into the trimethylsilyl ester derivative, which was then analysed by gas chromatography—mass spectrometry on a glass-coil column (1 m × 3 mm i.d.) packed with 3% HI-EFF-8BP on 80/100 mesh Gaschrom-Q at a column temperature of 218°C [8].

**Extraction of bile acids from bile and their analysis by g.l.c. (Scheme 3)**

Bile acids in bile collected after gall-bladder contraction (B-bile, 0.1 ml) were extracted with Amberlite XAD-2 and treated by alkaline hydrolysis after solvolysis. Free bile acids were extracted with ether, and converted into methyl ester acetyl derivatives, which were then analysed by the procedure used for serum bile acid analysis.

**Results**

**Serum cholesterol, \( \gamma \)-GT and bilirubin**

The results of the routine clinical tests are shown in Table 1. There were no significant sex differences in the values of these tests among the patients. The mean value of serum total cholesterol level in the patients was significantly lower than that in the normal controls. The mean value of serum \( \gamma \)-GT activity in the patients was significantly higher than that in the normal controls. These results are in agreement with other reports concerning patients with MMD [9].

No significant difference in the serum bilirubin level was noted. This might deny the existence of bile obstruction in these patients, which, as a consequence, indicates that the behaviour of their serum bile acids did not appear to be a secondary change due to bile obstruction.

**Identification and quantitative determination of serum bile acids**

The total patterns of methyl ester acetyl derivatives from g.l.c. on OV-1 of the extract by hexane, and of the non-sulphated and sulphated fractions from the patients' serum bile acids, are shown in Fig. 1.

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**Fig. 1.** Gas-liquid chromatographs on OV-1 of methyl ester acetyl derivatives of (a) the extract with hexane, (b) the non-sulphated fraction and (c) the sulphated fraction, from the patients' serum bile acids. P, Peak.

The materials with the same retention time as peak (a-1) and (b-1) in Fig. 1(a) and 1(b) were eluted in fraction (I) by silicic acid column chromatography and showed the same mass spectra as shown in Fig. 2(a). It showed two prominent peaks (\( m/e \) 368 and 247), which agreed with two mass units lower than those (\( m/e \) 370 and 249) found in the electron impact-MS spectrum of \( 3 \beta \)-hydroxy-5-cholen-24-oic acid. On
the other hand, acetylated cholesterol showed the same spectrum (Fig. 2). This material thus seems to be cholesterol rather than the derivative of 3β-hydroxy-5-cholen-24-oic acid having a double bond in the side chain. The latter possibility has still to be examined.

Identification and quantitative determination of individual bile acids in a serum sample were
Bile acid abnormality in myotonic dystrophy

**TABLE 2. Analysis of serum bile acids in the control subjects and patients with myotonic muscular dystrophy**

Subject numbers are the same as those used in Table 1. The amount of each peak was tentatively calculated as a bile acid having the same detector response as DCA, CDCA, cholic acid, UDCA, HCA, β-MCA, LCA, DCA or UDCA, respectively, corresponding to peaks b-2, b-3, b-4, b-5, b-6, b-7, c-1, c-2 or c-3 in Fig. 1. tr., Trace amount (<0.01 pg/ml). N.S., Not significant; N.D., not detectable.

<table>
<thead>
<tr>
<th>Peak no. (Fig. 1)</th>
<th>1</th>
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<th>1-36</th>
<th>0-17</th>
<th>0-37</th>
<th>0-79</th>
<th>0-70</th>
<th>0-09</th>
<th>0-09</th>
<th>N.D.</th>
<th>5-50</th>
</tr>
</thead>
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<tr>
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<td>2-79</td>
<td>1-66</td>
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<td>0-40</td>
<td>0-58</td>
<td>0-59</td>
<td>0-20</td>
<td>0-11</td>
<td>N.D.</td>
<td>6-74</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2-27</td>
<td>1-90</td>
<td>0-17</td>
<td>0-52</td>
<td>0-78</td>
<td>0-99</td>
<td>0-22</td>
<td>0-09</td>
<td>N.D.</td>
<td>6-94</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1-39</td>
<td>2-69</td>
<td>0-90</td>
<td>0-86</td>
<td>0-68</td>
<td>1-65</td>
<td>0-03</td>
<td>0-06</td>
<td>N.D.</td>
<td>8-26</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2-32</td>
<td>1-35</td>
<td>0-30</td>
<td>0-36</td>
<td>1-61</td>
<td>1-25</td>
<td>0-35</td>
<td>0-22</td>
<td>N.D.</td>
<td>7-76</td>
</tr>
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<td>2-14</td>
<td>1-79</td>
<td>0-39</td>
<td>0-50</td>
<td>0-89</td>
<td>1-04</td>
<td>0-18</td>
<td>0-11</td>
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<td></td>
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<tr>
<td>±(SD)</td>
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<td>(0-55)</td>
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<td>(0-21)</td>
<td>(0-41)</td>
<td>(0-43)</td>
<td>(0-12)</td>
<td>(0-06)</td>
<td>(1-06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1-62</td>
<td>2-77</td>
<td>0-75</td>
<td>0-62</td>
<td>1-20</td>
<td>1-05</td>
<td>0-30</td>
<td>0-37</td>
<td>N.D.</td>
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<td>0-22</td>
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<td>0-60</td>
<td>1-25</td>
<td>1-09</td>
<td>0-26</td>
<td>0-22</td>
<td>N.D.</td>
<td>7-83</td>
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<td>(0-04)</td>
<td>(0-07)</td>
<td>(0-05)</td>
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<td>N.D.</td>
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<td>0-14</td>
<td>N.D.</td>
<td>7-26</td>
</tr>
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<td>(0-59)</td>
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<td>(0-18)</td>
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<td>(0-35)</td>
<td>(0-11)</td>
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<td>7-32</td>
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<td>1-33</td>
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<td>tr.</td>
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<td>N.D.</td>
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<td>6-45</td>
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<td>0-64</td>
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<td>1-61</td>
<td>1-04</td>
<td>0-86</td>
<td>tr.</td>
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<td>N.D.</td>
<td>0-13</td>
<td>6-62</td>
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<td>1-26</td>
<td>1-12</td>
<td>1-03</td>
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<td>N.S.</td>
<td>&lt;0-001</td>
<td>&lt;0-001</td>
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<td>—†</td>
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<td>—†</td>
<td>N.S.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Containing DCA only; † Obvious difference.

carried out by g.l.c. without any preliminary group separation by silicic acid column chromatography. The results are listed in Table 2. Significant differences between the control and patients are shown in the materials corresponding to the peaks described below.

**Peak (b-2) (Fig. 1b) and peak (c-2) (Fig. 1c).**

The materials of the peak (b-2) and the peak (c-2) with a retention time corresponding to that of DCA on gas chromatography was eluted in fraction II by silicic acid column chromatography and was identified as DCA by gas chromatography-mass spectrometry in both the controls and patients (Figs. 3 and 4).

The serum level of sulphated and non-sulphated conjugates of DCA in patients with adult type MMD was three times that in normal controls.

**Peak (b-4) (Fig. 1b).** By silicic acid column chromatography the material with the same retention time as cholic acid on g.l.c. was separated into two components. The major component was eluted in fraction IV and was identified as cholic acid by gas chromatography-mass spectrometry. The minor one eluted in fraction II could not be identified, but gas chromatographic–mass spectrometric analysis implied the presence of a similar steroid-nucleus structure to CDCA with a longer side chain [peak (2-3) in Fig. 3(b) and Fig. 5(c)]. Contamination by the unknown component in the peak (b-4) amounted to 22.6% in patients but only a trace in the controls.

**Peak (b-5) (Fig. 1b).** The material of peak (b-5) with a retention time corresponding to that of UDCA on gas chromatography was eluted in fraction II only by silicic acid column chromatography and was revealed as a mixture by data from the mass chromatogram [peak (b-5) in Fig. 6]. The control values of crude UDCA calculated as described below corresponded to those in the earlier work with the same analytical procedure [10].

There are significant correlations between the amounts of peak (b-5) and peak (b-3), peak (b-7) and γ-GT respectively in the seven patients as shown below.

Y: the amount of peak (b-5) was tentatively calculated as the bile acid having the same detector response as UDCA (μmol/l).
\[ y = 0.04 + 0.26x \quad (P < 0.01) \] 
(n = 7).

Y: the same Y as described above.
X: the amount of peak (b-7) was tentatively calculated as the bile acid having the same detector response as $\beta$-MCA ($\mu$mol/l).
Regression line: \[ y = 0.13 + 0.38x \quad (P < 0.05) \] 
(n = 7). Correlation coefficient: \[ r = 0.75 \quad (P < 0.01) \] 
(n = 7).

Y: the same Y as described above.
X: the amount of $\gamma$-GT (i.u./l).
Correlation coefficient: \[ r = 0.70 \quad (P < 0.1) \] 
(n = 7).

**Peak (b-7) (Fig. 1b).** Peak b-7 in Fig. 1(b) was analysed by gas chromatography-mass spectrometry and the following fragmentation pattern was obtained (Fig. 7); when the mass spectrum was recorded as its methyl acetate ester, nothing assumed to be the molecular ion (M) was found and two series of fragment ions were recorded, one at $m/e$ 504, 472 and 412 and the other at $m/e$ 504, 454 and 394. These could have possibly arisen from dihydroxymono-oxocholic acid (Fig. 7a). The mass chromatogram indicates that the characteristic fragment ions with $m/e$ 394, 412, 454 and 472 may have arisen from a single compound. This compound might have a structure different from that of ordinary bile acids, with a longer side chain, and might be more polar.
because it showed a prolonged retention time on gas chromatography with a column packed with 3% OV-1.

Detection of iso-UDCA and iso-CDCA in patients' sera

By t.l.c. on chloroform/acetone/methanol (110:35:3, by vol.) UDCA, 3β,7β-dihydroxy-5β-cholan-24-oic acid and 3β,7α-dihydroxy-5β-cholan-24-oic acid could not be separated but the combined spot could partially be separated from DCA and totally separated from CDCA.

The scraped zone corresponding to standard UDCA, from t.l.c. of fractions I and II (ethanol/chloroform, 2–4:98–96, v/v) obtained by silicic acid chromatography of serum bile acids from the patients and the controls, was analysed by gas chromatography–mass spectrometry as described below (Figs. 8 and 9). The patients'
FIG. 5. Mass spectra of methyl ester acetyl derivatives of authentic CDCA and fraction of the sample. (a) Authentic CDCA methyl ester acetyl derivatives; (b) peak (2-2) in Fig. 3(b) of the patients' serum; (c) peak (2-3) in Fig. 3(b) of the patients' serum.

FIG. 6. Mass chromatogram of Fig. 3(a). The retention time of peak (b-5) in Fig. 6 corresponds to that of peak (b-5) in Fig. 1(b). P, Peak.
Bile acid abnormality in myotonic dystrophy

Fig. 7. Unidentified bile acids in the patients' sera. (a) Mass spectrum of the peak (2-7) of Fig. 3(b), i.e. peak (b-7) in Fig. 1(b). (b) Mass chromatogram of the peak (2-7) of Fig. 3(b), i.e. peak (b-7) in Fig. 1(b).

specimens consisted of four components, from peak (I) to peak (V). Peak (I), having the same retention time relative to deoxycholic acid (RRT) (0.83) as $3\beta,7\alpha$-dihydroxy-5$\beta$-cholan-24-oic acid, showed the base peak at m/e 370 (M-2 × 90) and peaks at m/e 460 (M-90) and m/e 255 (ABCD ring). The peaks at m/e 243, 249 and 262 are characteristic of a 3,7-bis(trimethylsiloxy) structure in a dihydroxy bile acid. As a result, peak (I) was taken to be $3\beta,7\alpha$-dihydroxy-5$\beta$-cholan-24-oic acid. Peak (II) was identified as DCA by gas chromatography—mass spectrometry and RRT (1.00) on g.l.c. Peak (IV) was $3\beta,7\beta$-dihydroxy-5$\beta$-cholan-24-oic acid and peak (V) was UDCA. In the control specimen, the material corresponding to $3\beta,7\alpha$-dihydroxy-5$\beta$-cholan-24-oic acid was nonexistent and even the fragment ion (m/e 370) was not detected at the RRT corresponding to $3\beta,7\alpha$-dihydroxy-5$\beta$-cholan-24-oic acid.

Identification and quantitative determination of biliary bile acids

The analysis of binary bile acids was performed by the procedure used for serum analysis, without separation of bile acids into sulphate-conjugated and non-sulphate-conjugated fractions. The total patterns on g.l.c. are shown in Fig. 10. The results of the quantitative analysis are listed in Table 3. The content of ursodeoxycholic acid in bile from one normal person determined by our analytical procedures corresponded to the average value for Japanese
subjects reported by Igimi [11]. In three patients the content of ursodeoxycholic acid was remarkably decreased.

Discussion

It has been suggested that MMD may be the expression of a cholesterol metabolic defect, perhaps analogous to experimental inhibition of cholesterol synthesis [1].

On analysis of serum from seven patients with adult type MMD, one of the unusual bile acids, corresponding to peak (b-7) in Fig. 1(b), correlated quantitatively with the material corresponding to the peak (b-5) (correlation coefficient 0.75, \( P < 0.05 \)). This might indicate an abnormality of bile acid metabolism in patients with MMD, with relative impairment in the degradation of the cholesterol side chain resulting in accumulation of dihydroxymono-oxycholanic acid with a longer side chain (peak 2-7 in Fig. 3(b), and dihydroxycholanic acid having a similar steroid-nucleus structure to chenodeoxycholic acid with a longer side chain (peak 2-3 in Fig. 3(b)).

In the peak (b-5) in the patients’ sera, 3β,7α-dihydroxy-5β-cholan-24-oic acid was detected, besides 3β,7β-dihydroxy-5β-cholan-24-oic acid and UDCA. Ordinarily 3β,7α-dihydroxy-5β-cholan-24-oic acid has been found only in human urine and faeces, and not in serum [12].

Although the total amount of serum bile acids is within the normal limit, an unusual composition of bile acid constituents is apparent in the serum of patients, i.e. increased DCA and decreased crude cholic acid and UDCA fractions on g.l.c. Such an irregularity in the pattern of serum bile acid composition has not been reported.

In this study a distinct difference was revealed between the patients and the controls in respect of serum level of DCA. The patients’ serum DCA was approximately three times that of the control, although the patients’ serum LCA, unlike DCA, was near the level of that of the controls. This cannot be accounted for by 7α-dehydroxylation of intestinal flora as a result of constipation [13]. Back [14, 15] has demonstrated the primary biosynthesis of DCA in human liver through an alternative pathway of cholesterol catabolism under pathological conditions. His findings imply the existence of such an alternative pathway in MMD.

With regard to the influence of the increased level of serum DCA, it is known that the effect of sodium DCA on the degradation of arachidonyl-labelled phosphatidylinositols by brain synaptosomes shows optimal activity [16]. This may suggest a reason why a child with mental retardation might be born from the mother with MMD.

As compared with the control, the crude UDCA concentration in every patient’s serum and bile was significantly lower. As to the
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Fig. 9. Mass spectra of the material corresponding to each peak in Fig. 8.
K. Tanaka, K. Takeshita and M. Takita

Fig. 10. Gas–liquid chromatography on OV-1 of methyl ester acetyl derivatives of biliary bile acids from (a) the control, and (b), (c) and (d) the patients.

biological action of UDCA, Kimura [17] has shown that the cytotoxicity of CDCA was decreased after preincubation with UDCA or by addition of UDCA and CDCA in the media. Greim et al. [18] have explained that the increase of UDCA biosynthesis in cholestasis could be considered a protective mechanism to prevent accumulation of strongly detergent bile acid likely to injure cell membranes. In contrast to such an effect of UDCA, DCA is cytotoxic to human cells [19]. This study revealed that the ratio of DCA to crude UDCA in patients’ serum was ten times that in control serum. These mutually reinforced actions of DCA and UDCA against human cells in MMD may account for the involvement of tissues other than skeletal muscle.

After UDCA is administered intravenously, electron microscopy shows that the Golgi apparatus within the pancreatic islet cells is enlarged and the exocrine secretion is remarkably stimulated [20]. This could be interpreted as indicating that UDCA affects the functions of the Golgi apparatus, which are the synthesis of membrane glycoprotein, the recycling of plasma...
membrane and secretion. If so, the findings of decreased levels of UDCA in patients’ serum and bile may have a bearing on their abnormal insulin secretion [21], and the abnormalities of glycoprotein of erythrocyte membranes from the patients [22]. From the viewpoint of the possible effect of UDCA on the function of Golgi apparatus, disturbances in recycling the plasma membrane, assumed to be caused by the decreased function of UDCA, may form the pathological basis of this generalized progressive disease, which is usually assumed to be due to generalized membrane abnormalities.

On the other hand, some aspects of the disease may be caused by the epimers of bile acids, i.e. 3β,7α-dihydroxy-5β-cholan-24-oic acid, which is normally eliminated in the urine and is not detectable in serum. Because an epimer of a structurally asymmetrical compound such as a bile acid will act with selective specificity in metabolism, the existence of various epimers of bile acids in patients, whether due to retention or to increased synthesis, might disturb bile acid metabolism in general.

It has been shown that bile acids increase the cellular membrane fluidity [23]. The abnormalities of bile acids in patients may be related to the increased fluidity of their erythrocyte membranes, which has been suggested as a cause of the clinical sign of myotonia in muscle [24]. As to the mechanism of muscular contraction, Shimizu & Yano [25] showed the importance of the environmental fluidity around the interaction between actin and myosin, whereby molecular reactions are transformed into an oriented tissue response, i.e. a chemical process changing to a mechanical process. Thus the detergent abnormalities of bile acids may account in some degree for the abnormalities in patients’ muscular contraction.

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References


### Table 3. Analysis of biliary bile acids in the control subjects and patients with myotonic muscular dystrophy

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Total bile acids (mg/ml)</th>
<th>LCA (mg/ml)</th>
<th>DCA (mg/ml)</th>
<th>CDCA (mg/ml)</th>
<th>Cholic acid (mg/ml)</th>
<th>UDCA (mg/ml)</th>
</tr>
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<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>F</td>
<td></td>
<td>4.7</td>
<td>38.4</td>
<td>29.1</td>
<td>27.0</td>
<td>0.8</td>
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<tr>
<td>1</td>
<td>29</td>
<td>F</td>
<td></td>
<td>2.6</td>
<td>18.9</td>
<td>40.2</td>
<td>40.1</td>
<td>1.2</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>M</td>
<td></td>
<td>3.3</td>
<td>19.2</td>
<td>39.8</td>
<td>36.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>(± SD)</td>
<td>3.5</td>
<td>24.5</td>
<td>36.4</td>
<td>34.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>3.6</td>
<td>15.8</td>
<td>34.4</td>
<td>40.3</td>
<td>5.9</td>
</tr>
<tr>
<td>Mean*</td>
<td></td>
<td></td>
<td>(± SD)</td>
<td>17.4</td>
<td>35.5</td>
<td>40.3</td>
<td>5.7</td>
<td>28.8</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
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<td>&lt; 0.001</td>
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</table>

* Control values from the data of Igimi [11].


