Catalase Activity in the Organs of Japanese Acatalasemias

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OGATA, M., MIZUGAKI, J. and TAKAHARA, S. Catalase Activity in the Organs of Japanese Acatalasemias. Tohoku J. exp. Med., 1974, 113 (3), 239-243 — The subjects of this study were a hereditary typical acatalasemia and a hereditary atypical acatalasemia. In the latter case, the children did not show hypocatalasemia but one of the cousins proved to be hypocatalasemia. The abdominal muscle and the vermiform appendix from the case of typical acatalasemia had only a minute catalase activity. The liver from the case of atypical acatalasemia indicated a moderate catalase activity and catalase protein was observed in it. ——— acatalasemia; tissue catalase activity

Acatalasemia was found to be a congenital constitutional abnormality with only minute catalase activity in blood and bone marrow (Takahara 1952). As to catalase activity of other organs, Nakamura et al. (1952) reported that organs (liver, muscle and bone marrow) of acatalasemia indicated no catalase activity by the Euler and Josephson method (1927). Aebi et al. (1964) reported that in Swiss acatalasemia, catalase activity was found to be 5.2% of the normal value in lymphoepithelial tissue but no activity was detected in liver tissue by U.V. spectrophotometry. Takahara (1968) reported that catalase activity was recognized in various organs from atypical acatalasemia by the manometric method.

In this experiment, catalase activity in organs from typical and atypical acatalasemia, and catalase protein in liver tissues from atypical acatalasemia were examined.

Materials and Methods

The typical acatalasemia blood sample employed in this study was obtained from the patient, K.N., whose genotypic and phenotypic characterizations of the biochemical defect had been studied thoroughly. A small piece of the abdominal muscle (the obliquus externus abdominis) and vermiform appendix were taken at the time of a surgical operation. Tissues of the atypical acatalasemia (M.O.) were taken from autopsy samples.

Human blood catalase purified by the method of Herbert and Pinsent (1948) was used for antibody formation. In turn, liver catalase extraction for antigen was carried out using a method modified after Nishimura et al. (1962). The liver homogenate was centrifuged and the supernatant solution was mixed and shook with approximately one-tenth volume of 5:1 ethanol-chloroform mixture. After centrifugation, 95% ethanol (-10°C) was added to the supernatant solution, and the resultant precipitate was dialyzed against saline at

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TABLE 1. Comparison of catalase activity in organs from normal and typical acatalasemia individuals measured by the titration method (Cat k/dry weight)

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th></th>
<th>Typical acatalasemia</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity</td>
<td>O/B</td>
<td>Activity</td>
<td>A/N</td>
</tr>
<tr>
<td>Blood</td>
<td>89.29</td>
<td>100</td>
<td>UDS</td>
<td></td>
</tr>
<tr>
<td>Vermiform appendix</td>
<td>11.30</td>
<td>12.7</td>
<td>0.30</td>
<td>2.7</td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td>2.08</td>
<td>2.3</td>
<td>UDS</td>
<td></td>
</tr>
</tbody>
</table>

O/B: Ratio of catalase activity in each organ to blood (%).
A/N: Ratio of catalase activity in acatalasemia organs to normal organs (%).
UDS: Under detectable sensitivity.

Estimation of catalase activity was carried out by the titration method of Euler and Josephson (1927) with the reaction mixture of 0.02N H₂O₂, 40 ml, phosphate buffer solution (pH 7.0), 10 ml, and enzyme solution, 1.0 ml, at 37°C. Reaction was stopped by addition of 5 ml of 2N H₂SO₄. Catalase activity was also estimated by the manometric method of Fujita and Kodama (1930). That is, 2 ml of tissue homogenate and 1 ml of 0.01 M phosphate buffer solution (pH 6.8) were placed into the main compartment of a Warburg's cup. QO₂ of catalase activity was expressed as the volume of oxygen released in 30 minutes at 37°C.

Immunodiffusion was carried out by the method of Ouchterlony (1953) with a disk (4 cm in diameter) made of 1% agar (Specific noble agar, Difco) in phosphate buffer solution (pH 7.0) containing 0.01% merthiolate. The central well and six circumferential wells were separated so that the distance from the edge of the central well to that of each circumferential well was about 3 mm. These wells were 5 mm in diameter. Antibody and antigens (0.04 ml each) were placed in the central well and circumferential wells, respectively.

RESULTS

As shown in Table 1, the vermiform appendix from the typical acatalasemia showed a minute catalase activity (2.7%) by the titration method, and in the abdominal muscle, catalase activity was under detectable sensitivity. On the other hand, the ratio of catalase activity in the vermiform appendix to that in blood was 4.5% by the manometric method. Catalase activity in the fresh liver from the atypical acatalasemia was also proved by the manometric method (Table 2), indicating that the ratios of catalase activity in acatalasemia liver and blood to normal ones were 38.6% and 4.0%, respectively. Subsequently, catalase protein in liver tissues of the atypical acatalasemia was proved by the double diffusion method (Fig. 1). One precipitin line of liver extract (crude catalase protein) from the atypical acatalasemia was fused with the precipitin line of normal liver and blood extracts, and purified human blood catalase. After antigen-antibody reaction, washing with saline and pouring 5% of H₂O₂ on the agar plate at 4°C, the bubble formation indicating catalase activity remained in the precipitin lines. However, another line, which was faint and near the catalase line, was recognized in the liver extract from the atypical acatalasemia.
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**TABLE 2.** Comparison of catalase activity in blood and liver from normal and atypical acatalasemia individuals measured by the manometric method (Qo₂/dry weight)

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Atypical acatalasemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity</td>
<td>O/B</td>
</tr>
<tr>
<td>Blood</td>
<td>6210</td>
<td>100</td>
</tr>
<tr>
<td>Liver</td>
<td>3845</td>
<td>62</td>
</tr>
</tbody>
</table>

O/B: Ratio of catalase activity in each organ to blood (%).
A/N: Ratio of catalase activity in acatalasemia organs to normal organs (%).

**DISCUSSION**

Our results indicated that a typical acatalasemia had a minute catalase activity in the vermiform appendix, but that the activity was not detectable in the abdominal muscle. On the other hand, as shown in Table 3, a moderate catalase activity was recognized in the small and large intestines and abdominal muscle by Takahara (1968). The vermiform appendix is similar in histological constitution to the rest of the intestine, having smooth muscle layers, lymph nodes and glands of Lieberkühn (Bloom and Fawcett 1968). Therefore, the presence of a minute catalase activity in the vermiform appendix suggests the presence of similar activity in other parts of the intestine.
TABLE 3. Comparison of catalase activity in several organs from normal and atypical acatalasemia individuals measured by the manometric method \((QO_2/dry weight)\) (Takahara 1968)

<table>
<thead>
<tr>
<th>Normal Activity</th>
<th>O/B</th>
<th>Atypical acatalasemia Activity</th>
<th>O/B</th>
<th>A/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>6210</td>
<td>100</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>Small intestine</td>
<td>500</td>
<td>8.0</td>
<td>155</td>
<td>62</td>
</tr>
<tr>
<td>Large intestine</td>
<td>550</td>
<td>8.5</td>
<td>160</td>
<td>64</td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td>325</td>
<td>130</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

O/B: Ratio of catalase activity in each organ to blood (%).
A/N: Ratio of catalase activity in acatalasemia organs to normal organs (%).

In liver tissues from the atypical acatalasemia, catalase activity and protein were recognized. Then, the catalase line was produced along with a faint precipitin line which was not present in normal liver tissues.

Nakamura et al. (1952) noted that the muscle or the liver tissue from the typical Japanese acatalasemia indicated no catalase activity and that several organs from Swiss cases of acatalasemia had minute catalase activity as reported by Aebi et al. (1964). In contrast to this, organs of radiation-induced mouse acatalasemia were proved to have a moderate catalase activity (Feinstein et al. 1967), and the present experiment showed that a minute activity was recognized in the vermiform appendix in Japanese acatalasemia.

From the above findings, acatalasemias were classified into two types; one with a minute catalase activity in the whole body —acatalas(em)ia— as described by Nakamura et al. (1952), Aebi et al. (1964) and as the case of K.N. in this study, and the other with a minute catalase activity only in the blood, such as the case of M.O. described above or an acatalasemia mouse.

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References

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