Deoxyribonucleic Acid Content of the Regenerative Nodule Cells in Liver Cirrhosis

I. OBSERVATIONS ON THE REGENERATIVE NODULE CELLS IN AN EXPERIMENTAL LIVER CIRRHOSIS

Osamu Yokoi

The First Department of Internal Medicine Okayama University Medical School
(Director: Prof. Kiyowo Kosaka)

The regenerative nodule in experimental rat cirrhosis induced by a choline-deficient diet is defined as a cell mass projected on the surface of the fibrous liver. On microscopic examination, the cell mass is consisted of small non-fatty or less fatty cells aligned on one cell thick plate. They are the cells of homogenous in the property with reduced succinic hehydrogenase activity. Occasionally another type of the regenerative nodules is also encountered. The nodules are consisted of the cells of not homogeneous in the property. The large cells having the large nuclei with more than two nucleoli are arranged in rather odd fasion, having a structure of adenoma. Both types of the regenerative nodules are formed in fatty liver cirrhosis and proliferate expansively. Customarily, such newly formed cell mass has been designated as the "regenerative" nodule. However, the mechanism operating the proliferation of the cells has recently debated by some investigators. Comparative studies have been attempted in connection with the study of hepatic cell proliferation after partial heptectomy in the normal liver by using a cytochemical method, tracing method using radioisotope, autoradiography using tritiated thymidine, however, no report came out to date for the elucidation of the subject using microspectrophotometry. The results obtained here were based on a study applied a microspectrophotometric method for the determination of the deoxyribonucleic acid (subsequently abbreviated as DNA) content of both the cells of regenerative nodules and internodular areas.

MATERIALS AND METHODS

Male, white rats of Sprague-Dawley strain weighed approximately 100 grams served for the present study. The animals were kept in an individual cage placed in a room of constant temperature and fed a choline-deficient diet termed C-8 (Table 1), 8 grams of it per day, for 203 to 478 days. The 10 experimental animals reached to the state of nodular cirrhosis (Hoffbauer IV) were selected for the study. Individual protocols about the experimental animals were summarized in Table 2. Longitudinal slices of liver tissue obtained from the left lateral lobe of the cirrhotic rats were immediately fixed in 50% formalin fixatives, and 10μ paraffin sections were placed on a coverglass of 24×50 mm in size with a thickness of 0.13~0.17 mm. The sections were hydrolyzed with N HCl at 60°C for 5 minutes, stained with Feulgen reagent for 4 hours, washed three times for 15 minutes respectively, in a mixture of 9 volumes Sorenson's 0.1 N glycine buffer of pH 2.28 and 1 volume 15% sodium metabisulfite. After the Feulgen stain, the specimen was covered by a coverglass interposed with Canada balsam of 1.7 refractory rate. Then, the DNA content of the cell nuclei was measured under a microspectrophotometer made by the Olympus Optical Company, Japan by using two-wavelength method which was developed by Ornstein and, independently, by Patau, and the correction tables of Mendelsohn was used for calculating the DNA content. For histologic study, longitudinal slices of liver tissue obtained from the left lateral lobe of the cirrhotic rats were fixed in alcoholic Bouin solution, and paraffin sections were cut 6μ thick and stained with hematoxylin and eosin.
Table 1. Hypolipotrophic Diet: C-8

1. Casein .................................... 8.00
2. Lard .................................... 37.95
3. Sucrose ................................. 48.375
4. Salts .................................... 4.00
5. Cystine ................................. 0.625
6. Vitamine powder ..................... 1.00
7. ADT (in oil) ........................... 0.05

Total .................................... 100.00

1. Vitamin-free test casein
2. Commercial
3. Commercial
4. Salt mixture No. 2, U.S.P.XIII
   - Calcium biphosphate 13.6%
   - Calcium lactate 32.7
   - Magnesium sulfate 13.7
   - Potassium phosphate 24.0
   - Sodium biphosphate 8.7
   - Sodium chloride 4.4
   - Ferric citrate 3.0

   Total 100

5. L-cystine
6. Vitamins, crystalline, in powdered sugar
   - Thiamine .................................... 0.3125 Gm.
   - Riboflavin ................................. 0.5000
   - Pyridoxine ................................. 0.3125
   - Calcium pantothenate .................. 1.2500
   - Nicotinic acid .............................. 1.2500
   - Menadione U.S.P. ........................ 0.3125
   - Powdered sugar ......................... 996.0625

7. Vitamins A and D plus tocopherol, in oil
   - 6.25000 mg. vitamin A (200,000 units/gm.-oil)
   - 0.78125 mg. vitamin D (400,000 units/gm.-oil)
   - 25.00000 mg. dl-Alpha tocopherol (oil)
   - 17.96825 mg. peanut oil

   Total 50.00000 mg.

Table 2.

<table>
<thead>
<tr>
<th>Case</th>
<th>Days on diet</th>
<th>Body weight (gr.)</th>
<th>Liver weight (gr.)</th>
<th>L. weight (%)</th>
<th>Autopsy findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>208</td>
<td>364</td>
<td>19.0</td>
<td>5.2</td>
<td>hypertrophic fatty nodular cirrhosis</td>
</tr>
<tr>
<td>No. 2</td>
<td>376</td>
<td>312</td>
<td>12.5</td>
<td>4.0</td>
<td>same above</td>
</tr>
<tr>
<td>No. 3</td>
<td>375</td>
<td>162</td>
<td>10.3</td>
<td>6.4</td>
<td>same above expect for the size of nodule was 1~5 mm.</td>
</tr>
<tr>
<td>No. 4</td>
<td>376</td>
<td>192</td>
<td>15.0</td>
<td>7.7</td>
<td>same above</td>
</tr>
<tr>
<td>No. 5</td>
<td>376</td>
<td>204</td>
<td>12.1</td>
<td>5.9</td>
<td>same above, ascites (+)</td>
</tr>
<tr>
<td>No. 6</td>
<td>203</td>
<td>128</td>
<td>7.7</td>
<td>6.0</td>
<td>same above, ascites (−)</td>
</tr>
<tr>
<td>No. 7</td>
<td>371</td>
<td>193</td>
<td>12.7</td>
<td>6.6</td>
<td>same above</td>
</tr>
<tr>
<td>No. 8</td>
<td>478</td>
<td>166</td>
<td>11.1</td>
<td>6.7</td>
<td>same above</td>
</tr>
<tr>
<td>No. 9</td>
<td>251</td>
<td>164</td>
<td>11.7</td>
<td>7.1</td>
<td>same above</td>
</tr>
<tr>
<td>No. 10</td>
<td>268</td>
<td>148</td>
<td>9.2</td>
<td>6.2</td>
<td>same above</td>
</tr>
</tbody>
</table>