NOTE

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IRON INCORPORATION INTO LIVER CELLS AND FERRITIN OF TUMOR-BEARING RATS

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Measurement of the incorporation of iron into liver cells and liver ferritin of the rat revealed that both incorporated less iron under tumor-bearing condition than in the normal state.

A pronounced change in iron metabolism occurs in tumor-bearing animals. In particular, a number of reports have discussed changes in ferritin.6,7,14) Tumor-bearing animals possess ferritin in their liver and cancer cells, which has an isoelectric point more acidic than that of the ordinary ferritin9) and which is reported to be identical to the ferritin of a fetus.1) The ferritin which appears in tumor-bearing animals contains a significantly less iron than the normal ferritin.10) There have been several reports concerning the incorporation of iron into ferritin4,5); the presence of ATP and ascorbic acid increased the incorporation of iron.8,12,13) We have examined the incorporation of iron into liver cells and liver ferritin of the rat.

Methods and Materials

Animals and Tumors: Male rats of the Donryu strain, weighing approximately 120 g, were used. The tumors employed were sarcoma induced by 4-nitroquinoline 1-oxide (4-NQO) and ascites hepatoma AH-62F. Approximately 0.5 g of 4-NQO sarcoma tissue was transplanted on the back of a rat. Tests were conducted 30 to 40 days after the transplantation at which time the ratio of tumor weight to rat weight had reached 30-40%. In the case of AH-62F, approximately 0.2 ml of the ascites was injected into the abdominal cavity of a normal rat. Tests were conducted 10-15 days after the administration.

Rat Liver Ferritin: Rat liver ferritin was purified by the Granick method.8)

Anti-rat Ferritin Serum: Anti-rat ferritin serum was prepared by injecting a rabbit with 10 mg of the purified ferritin in complete Freund's adjuvant. The anti-rat ferritin serum obtained after 45 days contained, in 1 ml, the amount of the antibody sufficient to precipitate 500 µg of ferritin.

Ferritin Precipitation Technique: The following steps were performed at 2°C. Ammonium sulfate was added to the liver homogenate. The precipitate obtained at 50% of saturation was collected by centrifugation, dissolved in 10 ml of water, and the resulting solution was again centrifuged at 10,000 rpm for 1 hr. The supernatant was then added with 3 ml of the anti-rat ferritin serum and the mixture was stored overnight at 4°C. The mixture was centrifuged and the precipitate was suspended in cold 0.9% NaCl and centrifuged. This operation was repeated two more times, after which the precipitate was washed with cold 0.9% NaCl.

Radioactive Iron: 59Fe was employed in the form of ferric citrate procured from the Radiochemical Centre, England.

Results and Discussion

Incorporation of 59Fe into Cell and into Ferritin in Liver Slice: Liver slices from normal and tumor-bearing rats were tested for the 59Fe incorporation into the cells and ferritin. A slice of liver tissue (2.2 g), obtained by a slicer, was incubated with Krebs-Ringer phosphate buffer (pH 7.2) and 59Fe (50,000 cpm) for 1 hr at 37°C. Incorporation of 59Fe into ferritin in a slice proceeded linearly for 1 hr, after which the rate progressively de-

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Y. MAKINO AND K. KONNO

Table I. Incorporation of $^{59}$Fe into Liver Cells and Ferritin

<table>
<thead>
<tr>
<th>Animals bearing</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
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<tbody>
<tr>
<td></td>
<td>Cell (A)</td>
<td>Ferritin (B)</td>
<td>B/A</td>
</tr>
<tr>
<td>None</td>
<td>2000</td>
<td>190</td>
<td>0.086</td>
</tr>
<tr>
<td>4-NQO sarcoma</td>
<td>1365</td>
<td>28</td>
<td>0.015</td>
</tr>
<tr>
<td>AH-62F</td>
<td>1300</td>
<td>54</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Table II. Incorporation of $^{59}$Fe into Ferritin of Liver Homogenate

<table>
<thead>
<tr>
<th>Animals bearing</th>
<th>Incorporation of $^{59}$Fe (cpm/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 1</td>
</tr>
<tr>
<td>None</td>
<td>5878</td>
</tr>
<tr>
<td>4-NQO sarcoma</td>
<td>290</td>
</tr>
<tr>
<td>AH-62F</td>
<td>3236</td>
</tr>
</tbody>
</table>

creased. Upon completion of the incubation, the slice was washed three times with 0.9% NaCl and the following operations were then performed at 4°C. The slice was homogenized with 11 ml of 0.01M NaCl, and the radioactivity of a portion of the homogenate was measured. This value was taken as the amount of $^{59}$Fe incorporated into the cells. Ferritin was then precipitated from the homogenate and $^{59}$Fe incorporated into ferritin was measured. These results are shown in Table I. Saltman et al.\textsuperscript{13} reported that the cell membrane has no potential energy barrier to passage of iron into the cell, and Mazur et al.\textsuperscript{12} reported that ATP is related to the transfer of iron ions into the cells. Our results suggest that the number of iron ions which are transported into the cells through the liver cell membrane is smaller in tumor-bearing rats than in normal rats. The difference may have arisen from some inhibitory change in the permeation mechanism for iron ion of the liver cell membrane of the tumor-bearing rat, or it may be due to the lack of requirement for iron within the cells. The proportion of liver cell iron which is combined with ferritin is substantially lower in the tumor-bearing rat than in the normal rat\textsuperscript{10}, even though the total amount of iron in the liver cell in the tumor-bearing rat is much lower than that in the normal animal. Table I also shows that the ratio of the iron combined to ferritin to the total cellular iron (B/A) is lower in the tumor-bearing than in the normal rat.

Incorporation of $^{59}$Fe into Liver Homogenate Ferritin: The incorporation of $^{59}$Fe into the ferritin of liver homogenate was determined for normal and tumor-bearing rats. After homogenization of 1 g of rat liver with 10 ml of 0.15M KCl-0.0006M KHCO$_3$, 0.1 ml of $^{59}$Fe (25,000 cpm) was added to 5.0 ml of the homogenate. The mixture was then incubated for 30 min at 37°C, processed as described, and its radioactivity was measured. The results obtained are shown in Table II. The amount of $^{59}$Fe incorporated into liver ferritin is smaller for tumor-bearing rats than for normal rats. This is especially true with rats bearing 4-NQO sarcoma. The amount of ferritin in the liver of tumor-bearing rats is similar to that in normal rats\textsuperscript{11} but the amount of iron bound to ferritin is smaller\textsuperscript{10} in the tumor-bearing animals. The lower incorporation of $^{59}$Fe observed for tumor-bearing rats may indicate that these rats have either smaller number of the iron-ferritin binding sites\textsuperscript{4,5} or only insufficient amount of ATP, ascorbic acid, or others which have been shown by Mazur et al.\textsuperscript{12} and Miller et al.\textsuperscript{13} to be required for iron incorporation.

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IRON INCORPORATION INTO FERRITIN

REFERENCES

11) Makino, Y., unpublished data.