STUDIES ON ZINC-METABOLISM OF TUMOR. II.
BEHAVIORS OF \(^{65}\text{Zn}-\) AND \(^{203}\text{Hg}-\)HEMATOPORPHYRINS INJECTED INTO EHRLICH ASCITES TUMOR-BEARING MOUSE*

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INTRODUCTION

When the \(^{65}\text{Zn}\)-glycine complex was injected into the Ehrlich ascites tumor-bearing mouse, \(^{65}\text{Zn}\) was found by the authors to be accumulated into the tumor cells and to be maintained for a fairly long time in the course of proliferation of the cells\(^1\)). These phenomena are very interesting in relation to Fujii's hypothesis that the zinc plays an important role in the mitosis of cells\(^2\)). From the fact that the \(^{14}\text{C}\)-labeled glycine itself was accumulated into the tumor cells\(^3\)), however, the question arises whether \(^{65}\text{Zn}\) can be incorporated or not into the cells in only the form of glycine complex, that is to say, whether \(^{65}\text{Zn}\) has or not the affinity to the tumor cells in any other chemical form. On the other hand, the accumulation of the injected porphyrin was found in the tumor tissues by Auler\(^4\)) and Figge et al.\(^5\)) Considering this fact, it is expected that the tumor cells might take up more of the zinc attached to the hematoporphyrin ring in the same way as the zinc-glycine complex. In the present paper, it is shown that the behavior of \(^{65}\text{Zn}\)-hematoporphyrin injected into the tumor-bearing mouse is contrary to the above expectation and a factor which controls the uptake of zinc (generally, of metal) by the tumor cells is discussed by comparing the behaviors of \(^{65}\text{Zn}\)-glycine complex and \(^{65}\text{Zn}-\) and \(^{203}\text{Hg}\)-hematoporphyrins.

EXPERIMENTAL METHODS

Animals and Tumor: The Ehrlich ascites tumor was transplanted in the inbred

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ddN strain of male and female mice, each of which has about 20 g of body weight. Transplantations were always done by intraperitoneal inoculation with about 0.1 ml of seven day-old tumor ascites fluids.

**Preparations of Isotope Solutions:**

(a) $^{65}$Zn-hematoporphyrin solution: 50 mg of $^{65}$Zn-hematoporphyrin-Na$_2$ was dissolved in 20 ml of distilled water. This aq. solution contained 2.2 $\mu$c/ml of radioactivity.

(b) $^{203}$Hg-hematoporphyrin solution: 40 mg of $^{203}$Hg-hematoporphyrin-Na$_2$ was dissolved in 20 ml of distilled water. The radioactivity of this aq. solution was estimated to be 7.39 $\mu$c/ml.

**Injections of Isotope Solutions:** The $^{65}$Zn- or $^{203}$Hg-hematoporphyrin solution was injected intraperitoneally or subcutaneously on the back of the mouse on the fifth day after the transplantation of tumor. As the control for the experiment using $^{65}$Zn-hematoporphyrin, the injections of the $^{65}$Zn-glycine complex solution containing 2.2$\mu$c/ml of radioactivity were made under the same conditions as the $^{65}$Zn-hematoporphyrin was injected.

**Preparations of Samples:** The animals were killed by cervical dislocations on the first, third and sixth days after the injection of the isotope solutions. The ascites fluids collected from the abdominal cavity were immediately centrifuged and the supernatant, namely, the ascites plasma was pooled as the sample for determination of radioactivity. The tumor cells as the sample were obtained from the recovered cells by washing with the hypertonic sucrose solution and then by recentrifuging. If the recovered cells contained erythrocyte, they were hemolyzed previous to the washing procedure. On the other hand, the liver, kidney and spleen were removed by blunt-dissection. In the case of $^{65}$Zn-hematoporphyrin, the above samples were digested with 5 ml of conc. sulfuric acid added a small amount of both potassium and copper sulfates and then their radioactivities were measured. In the case of $^{203}$Hg-hematoporphyrin, the loss of $^{203}$Hg in the course of wet-ashing with sulfuric acid forced us to adopt the following procedure. Previous to the digestion with conc. sulfuric acid, the ascites plasma, the tumor cells suspended in water, and the liver homogenate were provided as the samples for determinations of radioactivities.

**Determination of Radioactivity and Total Nitrogen:** As described in a previous paper, the radioactivity was measured with a scintillation counter and the total nitrogen was determined by the micro-Kjeldahl method.

**Experimental Results**

**Behaviors of $^{65}$Zn-Hematoporphyrin:** Fig. 1 shows the radioactivity in counts per min. per 100 mg of nitrogen found in each sample after the subcutaneous injection of 0.2 ml of the $^{65}$Zn-hematoporphyrin solution on the back of the mouse which is bearing
Fig. 1 The radioactivities recovered in Ehrlich ascites tumor cells, its ascites plasma, and the liver of the tumor-bearing mouse, which received subcutaneously 0.44 μc of 65Zn-hematoporphyrin on the fifth day after the inoculation of tumor.

Fig. 2 The radioactivities recovered in Ehrlich ascites tumor cells, its ascites plasma, and the liver of the tumor-bearing mouse, which received intraperitoneally 1.10 μc of 65Zn-hematoporphyrin on the fifth day after the inoculation of tumor.

Fig. 3 The radioactivities recovered in Ehrlich ascites tumor cells, its ascites plasma, and the liver of the tumor-bearing mouse, which received subcutaneously 1.48 μc of 203Hg-hematoporphyrin on the fifth day after the inoculation of tumor.

Fig. 4 The radioactivities recovered in Ehrlich ascites tumor cells, its ascites plasma, and the liver of the tumor-bearing mouse, which received intraperitoneally 1.48 μc of 203Hg-hematoporphyrin on the fifth day after the inoculation of tumor.
five day-old tumor. On the first day, the radioactivity was largely recovered in the ascites plasma, from which then fairly rapidly disappeared with the lapse of days. It was hardly, however, found in the tumor cells between the first and sixth days.

The results are shown in Fig. 2 where each mouse received intraperitoneally 0.5ml of the $^{65}$Zn-hematoporphyrin solution. In the ascites plasma, the radioactivity largely remained on the first day and then fairly rapidly decreased in the course of days. The tumor cells, liver and the other tissues tested have low radioactivities. While the activities slowly increase in the liver and spleen, those of the kidney and tumor cells slowly decrease.

**Behaviors of $^{203}$Hg-Hematoporphyrin:** In both cases of the subcutaneous and intraperitoneal injections of 0.2ml of the $^{203}$Hg-hematoporphyrin solution, the radioactivity in the tumor cells was largely recovered on the first day but diminished to nearly zero on the third day. In the liver and ascites plasma, the radioactivities were very low or nearly zero through the period from the first to sixth days. These results are shown in Figs. 3 and 4.

**DISCUSSIONS**

It was shown in a previous paper\(^1\) that the difference between the apparent and actual excretion coefficients of the radioactivity denoted by $\bar{a}$ and $a$, respectively, is equal to the specific rate coefficient of the increase of total nitrogen, $\mu$, that is,

\[ a - \bar{a} = \mu \]  

(1)

where

\[ a = -\frac{1}{S} \frac{dS}{dt} \]  

\[ \bar{a} = -\frac{1}{Z^*} \frac{dZ^*}{dt} \]  

\[ \mu = \frac{1}{N} \frac{dN}{dt} \]

($S$ = c.p.m./100 mg of nitrogen),

($Z^*$ = total c.p.m./whole tissue),

($N$ = total nitrogen/whole tissue).

These coefficients are generally functions of time, $t$. When the radioactivity, $S$, and the total nitrogen, $N$, after the injections of $^{65}$Zn-hematoporphyrin are rearranged in semi-logarithmic scale, the coefficients $a$ and $\mu$ are found to be constant and independent on time as shown in Figs. 5 to 8. The values of $a$ and $\mu$ calculated from the above data are summarized in Tables 1 and 2 with the value of $\bar{a}$ estimated by using Eqn. (1).

From the results of control experiments using $^{65}$Zn-glycine complex, the coefficients $a$, $\bar{a}$ and $\mu$ are calculated in the same way and summarized in Tables 3 and 4.

It can be clearly seen in Tables 3 and 4 that the $^{65}$Zn of zinc-glycine complex is accumulating in the tumor cells ($\bar{a} = -0.461$ and $-1.524$) in spite of the fact that the radioactivity is on the apparent decrease ($a = 0.264$) in the case of intraperitoneal injection. (See Fig. 3 in a previous paper\(^1\).)
Fig. 5 The apparent excretion of the radioactivities in the tumor cells, its ascites plasma and various tissues of Ehrlich ascites tumor-bearing mouse, which received subcutaneously 0.44\( \mu \)c of \(^{65}\)Zn-hematoporphyrin on the fifth day after the inoculation of tumor.

![Graph for Fig. 5](image)

Fig. 6 The increases of the total nitrogen of the tumor cells, its ascites plasma and various tissues of Ehrlich ascites tumor-bearing mouse, which received subcutaneously 0.44\( \mu \)c of \(^{65}\)Zn-hematoporphyrin on the fifth day after the inoculation of tumor.

![Graph for Fig. 6](image)

Fig. 7 The apparent excretions of the radioactivities in the tumor cells, its ascites plasma and various tissues of Ehrlich ascites tumor-bearing mouse, which received intraperitoneally 1.10\( \mu \)c of \(^{65}\)Zn-hematoporphyrin on the fifth day after the inoculation of tumor.

![Graph for Fig. 7](image)

Fig. 8 The increases of the total nitrogen of the tumor cells, its ascites plasma and various tissues of Ehrlich ascites tumor-bearing mouse, which received intraperitoneally 1.10\( \mu \)c of \(^{65}\)Zn-hematoporphyrin on the fifth day after the inoculation of tumor.

![Graph for Fig. 8](image)
On the other hand, the values of $\bar{\alpha}$ in Tables 1 and 2 show that the actual excretion of the radioactivity from the ascites plasma goes on at fairly high rate ($\bar{\alpha} = 0.348$ and 0.473) and that the activity of tumor cells does not much decrease or increase ($\bar{\alpha} = -0.057$ and 0.049).

It has been observed by Ely and Batt\(^6\) that the hematoporphyrin does not go within the living ascites tumor cells and can be adsorbed on the leucocyte, dead cells or cell

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**Table 1.** The specific rate of increase of total nitrogen and the apparent and actual excretion coefficients after the subcutaneous injection of $^{65}$Zn-hematoporphyrin.

<table>
<thead>
<tr>
<th></th>
<th>Tumor cells</th>
<th>Ascites plasma</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific rate of increase of N, $\mu$</td>
<td>0.001</td>
<td>-0.048</td>
<td>-0.050</td>
<td>-0.022</td>
<td>-0.068</td>
</tr>
<tr>
<td>Apparent excretion coefficient, $\alpha$</td>
<td>-0.056</td>
<td>0.300</td>
<td>-0.088</td>
<td>-0.061</td>
<td>-0.176</td>
</tr>
<tr>
<td>Actual excretion coefficient, $\bar{\alpha}$</td>
<td>-0.057</td>
<td>0.348</td>
<td>-0.038</td>
<td>-0.039</td>
<td>-0.108</td>
</tr>
</tbody>
</table>

**Table 2.** The specific rate of increase of total nitrogen and the apparent and actual excretion coefficients after the intraperitoneal injection of $^{65}$Zn-hematoporphyrin.

<table>
<thead>
<tr>
<th></th>
<th>Tumor cells</th>
<th>Ascites plasma</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific rate of increase of N, $\mu$</td>
<td>0.075</td>
<td>0.078</td>
<td>-0.003</td>
<td>0.006</td>
<td>-0.109</td>
</tr>
<tr>
<td>Apparent excretion coefficient, $\alpha$</td>
<td>0.124</td>
<td>0.551</td>
<td>-0.088</td>
<td>0.162</td>
<td>-0.214</td>
</tr>
<tr>
<td>Actual excretion coefficient, $\bar{\alpha}$</td>
<td>0.049</td>
<td>0.473</td>
<td>-0.085</td>
<td>0.156</td>
<td>-0.105</td>
</tr>
</tbody>
</table>

**Table 3.** The specific rate of increase of total nitrogen and the apparent and actual excretion coefficients after the subcutaneous injection of the $^{65}$Zn-glycine complex.

<table>
<thead>
<tr>
<th></th>
<th>Tumor cells</th>
<th>Ascites plasma</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific rate of increase of N, $\mu$</td>
<td>0.024</td>
<td>-</td>
<td>-0.062</td>
<td>-0.033</td>
<td>-0.151</td>
</tr>
<tr>
<td>Apparent excretion coefficient, $\alpha$</td>
<td>-0.437</td>
<td>-</td>
<td>0.263</td>
<td>0.142</td>
<td>0.104</td>
</tr>
<tr>
<td>Actual excretion coefficient, $\bar{\alpha}$</td>
<td>-0.461</td>
<td>-</td>
<td>0.325</td>
<td>0.175</td>
<td>0.255</td>
</tr>
</tbody>
</table>

*The coefficients cannot be obtained from the data with respect to the ascites plasma, because the graph of log S or log N versus time does not become a straight line.

**Table 4.** The specific rate of increase of total nitrogen and the apparent and actual excretion coefficients after the intraperitoneal injection of the $^{65}$Zn-glycine complex.

<table>
<thead>
<tr>
<th></th>
<th>Tumor cells</th>
<th>Ascites plasma</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific rate of increase of N, $\mu$</td>
<td>1.788</td>
<td>0.235</td>
<td>-0.038</td>
<td>-0.024</td>
<td>0.004</td>
</tr>
<tr>
<td>Apparent excretion coefficient, $\alpha$</td>
<td>0.264</td>
<td>0.073</td>
<td>0.136</td>
<td>0.071</td>
<td>0.160</td>
</tr>
<tr>
<td>Actual excretion coefficient, $\bar{\alpha}$</td>
<td>-1.524</td>
<td>-0.162</td>
<td>0.174</td>
<td>0.095</td>
<td>0.156</td>
</tr>
</tbody>
</table>
debris. Considering their observations, a low radioactivity of the tumor cells shown in Figs. 1 and 2 may be due to (1) the $^{65}$Zn-hematoporphyrin adsorbed on the tumor cells, which cannot be removed by washing, or to (2) the $^{65}$Zn liberated from the hematoporphyrin ring by hydrolysis or by substitution with the other metals and then incorporated into the tumor cells. According to the classification of metallo-porphins by Barnes and Dorough, the zinc-hematoporphyrin belongs to the group of small divalent metal complex. In this group, the metal (zinc) atom fits in the space surrounded by the four nitrogen atoms of porphin ring. The chemical bonds between zinc and nitrogen atoms, therefore, would be resistant to hydrolysis and replacement by the other metals, though, these covalent bonds have partly ionic nature. This fact disapproves the second possibility where the $^{65}$Zn incorporates into the tumor cells after the liberation from the porphyrin ring. The first possibility is also supported by the fact that the $^{65}$Zn-content of the tumor cells shows both inconsistent tendencies of increase and decrease and their values are nearly zero ($\bar{z} = -0.057$ and 0.049), contrary to the case of $^{65}$Zn-glycine complex. It may be thus concluded that $^{65}$Zn-hematoporphyrin does not accumulated in the tumor cells. The temporary high radioactivity of the ascites plasma is, however, considered to be due to the $^{65}$Zn-hematoporphyrin from the same point of view. This high radioactivity fairly rapidly decreases with the biological half-value period of a day or two. On the other hand, Figge et al. observed that the injected zinc-hematoporphyrin was accumulated in the tumor tissues of mice which are bearing the methylcholanthren-induced sarcoma and was maintained for a long time between ten and fourteen days. This difference of the behaviors of zinc-hematoporphyrin may be attributed to the difference of conditions in the ascites plasma and the intercellular fluids.

The behaviors of $^{203}$Hg-hematoporphyrin can also be explained from the same viewpoint. In the group of large divalent metal complex, which the mercury-hematoporphyrin belongs to, the size of metal atom is too large to fit easily into the space surrounded by the four nitrogen atoms. The mercury atom, then, juts out from the intramolecular space and are held mostly by coulombic forces with the nitrogen atoms. They are therefore unstable against the hydrolysis or the replacement reaction. This nature suggests that the mercury atom might be liberated from the hematoporphyrin ring and taken up by the tumor cells. This expectation is realized in Figs. 3 and 4. Contrary to the case of $^{65}$Zn-hematoporphyrin, the high radioactivity on the first day is referred to the $^{203}$Hg taken up by the tumor cells. The damage of tumor cells by $^{203}$Hg and their disintegrations may bring about the subsequent marked decrease of radioactivity accompanied with a decrease of the volume of packed tumor cells. In any case, it may be safely said that the uptake of metal (zinc or mercury) by the tumor cells is partly decided by the nature of the chemical bond, namely, the type of bond, its strength, and the stereochemistry or steric factor, etc.
between the metal atom and its ligands in the complex compound.

An inhibition of the growth of the tumor can be also seen when the $^{65}$Zn-hematoporphyrin solution was injected. As described in a previous paper\(^1\), the $\mu$ is considered to be equal to the specific rate of multiplication of cells provided that the nitrogen content per cell is constant throughout the life of the cell. In both cases where the injections were made subcutaneously and intraperitoneally, the value of $\mu$ of the tumor cells after the injection of $^{65}$Zn-hematoporphyrin is only a twenty-fourth part of that after the injection of $^{65}$Zn-glycine complex as shown in Table 5.

Table 5. The anti-tumor action of $^{65}$Zn-hematoporphyrin, assuming that the specific rate of increase of total nitrogen is equal to the specific rate of multiplication of cells.

<table>
<thead>
<tr>
<th>Specific rate of increase of N, $\mu$, in the case of</th>
<th>Subcutaneous injection 0.44$\mu$C</th>
<th>Intraperitoneal injection 1.10$\mu$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{65}$Zn-hematoporphyrin</td>
<td>0.001</td>
<td>0.075</td>
</tr>
<tr>
<td>$^{65}$Zn-glycin complex</td>
<td>0.024</td>
<td>1.788</td>
</tr>
<tr>
<td>Inhibition effect*</td>
<td>1/24</td>
<td>1/24</td>
</tr>
</tbody>
</table>

*The inhibition effect is defined as the ratio of the value of $\mu$ in the case of $^{65}$Zn-hematoporphyrin to that of $^{65}$Zn-glycine complex.

This result leads us to an idea of the "radio-dynamic action", that is, the damage of the living cells by the cooperation of hematoporphyrin with the radiation from $^{65}$Zn atom. This idea is supported by the experiment that the glycolytic metabolism of Ehrlich ascites tumor cells can be inhibited by the irradiation of X-rays in the presence of hematoporphyrin\(^8\). Still more investigations are, however, necessary for the establishment of a role of the radiodynamic action in the anti-tumor mechanism.

**SUMMARY**

The behaviors of $^{65}$Zn- and $^{203}$Hg-hematoporphyrins injected into Ehrlich ascites tumor-bearing mice were studied in comparison with that of the $^{65}$Zn-glycine complex and the following results were obtained.

1. In both cases of the subcutaneous and intraperitoneal injections of $^{65}$Zn-hematoporphyrin, the high radioactivity was found in the ascites plasma on the first day after the injections and then disappeared at fairly high rate with the lapse of days. A low or no radioactivity recovered in the ascites tumor cells did not so much vary in the course of days.

2. In both cases of the subcutaneous and intraperitoneal injections of $^{203}$Hg-hematoporphyrin, the high radioactivity was found in the tumor cells on the first day and then rapidly reduced to nearly zero on the third day after the injections. The
ascites plasma and the liver contained no radioactivity during the period from the first to sixth days.

3. These behaviors can be understood from the point of view that the uptake of metal (zinc or mercury) by the tumor cells may be controlled by the nature of the chemical bond between the metal atom and its ligands in the complex compound.

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REFERENCES