AGE AND SEX DIFFERENCES IN THE CLEARANCE OF INTRAVENOUSLY INJECTED COLLOIDAL CARBON FROM PERIPHERAL BLOOD IN RATS

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Abstract—Age and sex differences of carbon clearance from the peripheral blood of the rat were investigated. The clearance of carbon was more rapid in the young rats than in the old. Sex difference was significant in the rats of 10 and 14 weeks old, but not significant in the rats of 3 weeks old. Liver blood flow, extraction rate of carbon by the liver and whole circulating blood volume may be the factors involved in the sex and age differences of the blood clearance of carbon.

Key words: blood clearance, colloidal carbon, age difference, sex difference, liver blood flow, extraction rate

INTRODUCTION

In order to evaluate the toxicity of foreign particulate substances, such as bacteria or heavy metal fumes, it is important to know their movement after the uptake into the blood. A general model for the movement of particulate substances in the body and the relationship of the reticuloendothelial system (RES) function and blood clearance were showed by GABLIELI et al. (1965).

Colloidal carbon has been used as biological inert particles in many studies, especially in the study of RES (HALPERN et al., 1953; BIOZZI et al., 1953; NORMANN et al., 1965; FIKLINS et al., 1969; BILDER, 1976; BILDER, 1976; JOEL, 1978). In recent years, many kinds of radioactive particles were used, i.e., $^{131}$ I-latex particles (ADLERSBERG et al., 1969; SINGER et al., 1969), $^{198}$ Au-colloid (ZILVERSMIT et al., 1952; VETTER, 1954, 1956; YAKUSIJI, 1965; SINGER, 1972; MATSUOKA et al., 1972; ADLERSBERG et al., 1973), $^{131}$ I-macroaggregated albumin (BENACERRAF et al., 1957) and $^{99m}$ Tc-colloid.
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(DENALDO et al., 1976; KITANI et al., 1972) for the functional test of the RES or the liver and for imaging of organs. These studies indicated that the movement of particle was affected by the size, dose, chemical form and ionic surface charge of the particle as well as the coexisting stabilizing agents.

On the other hand, it is necessary to clarify the differences of animal species, strain, age and sex in the movement of particles in order to apply the results of animal experiment to human. BENACERRAF et al. (1957) reported the blood clearance of $^{131}$I-macroaggregated albumin in the mouse, rat, guinea-pig and rabbit to estimate the liver blood flow in each animal. BIOZZI et al. (1953) showed that the blood clearance of colloidal carbon was influenced by the relative weight of the liver and the spleen.

The object of this study is to investigate the sex and age differences of colloidal carbon clearance from the peripheral blood of the rat after i. v. injection and to make clear what physiological factors are responsible for the differences.

**MATERIALS AND METHODS**

*Animals*: Male and female rats, aged 3 to 68 weeks, were used. During the blood clearance experiment, they were anesthetized with nembutal given intraperitoneally at a dose of 2 mg per 100 g body weight. All animals were sacrificed by cutting the cervical artery after the experiment and weight of the liver and the spleen was measured.

*Colloidal carbon clearance*: Colloidal carbon (Günther Wagner C11/1431a) was used in this study. This carbon particles have a uniform diameter of about 300 Å and stabilized with fish gelatin. The original preparation, containing carbon at a concentration of about 100 mg/ml, was diluted two to five times with saline and used without any other treatment such as centrifugation or filtration. All rats were injected with above solution into the left femoral vein, and blood samples of 0.02 ml were obtained with heparinized glass capillary pipette by cutting the tail at scheduled times after injection. These blood samples were lysed in 2 ml of 0.1% Na$_2$CO$_3$ solution and the concentration of carbon was determined by spectrophotometer (Shimazu, D-40S) at a wave length of 660 μm.

*Clearance of $^{198}$Au-colloid*: Colloidal gold particles used in this study were obtained from Daiichi Radio Isotope Labs., Ltd, Tokyo. The specific activity of the solution is 10 –20 mCi per mg gold and radioactive concentration is 1 mCi per ml solution. These colloidal gold particles had a nearly identical size as compared with carbon particles and were stabilized with the same agents. To keep the concentration of gold and radioactivity constant, i. e., 100 μCi per ml, 0.025–0.075 mg gold per ml, the original solution was diluted with saline and nonradioactive colloidal gold solution before the experiment. The solution of 0.2 ml which contained 20 μCi, 0.005–0.015 mg gold per 100 g body weight was injected into the left femoral vein. Blood samples were collected by cutting the tail at scheduled times after injection and their activity was measured by Armac Scintillation Detector (Packard Model 440).

*Phagocytic index K*: Phagocytic index K, K-value, was calculated from the following
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equation:

\[ K = \frac{\log C_i - \log C_f}{t_1 - t_2} \]

where \( C_1 \) and \( C_2 \) are the concentration of colloidal carbon or \(^{198}\)Au-colloid at times \( t_1 \) and \( t_2 \).

*Extraction rate of colloidal carbon by the liver*: Male rats, aged 7 weeks, were injected with 5, 10 and 15 mg colloidal carbon per 100 g body weight, and rats of 17 weeks old were injected with 13 mg carbon per 100 g weight. The peripheral blood samples were collected at 2, 4 and 8 minutes after injection. At 8 minutes after injection, the blood of hepatic vein was collected. Extraction rate (ER) of colloidal carbon by the liver was calculated by the following equation:

\[ ER = \frac{C_P - C_I}{C_P} \]

where \( C_P \) is a concentration of colloidal carbon in the peripheral blood and \( C_I \) is that in the blood of hepatic vein.

**RESULTS**

*Dose effect of carbon on blood clearance*: The clearance of colloidal carbon in the rat after injection at various dose levels is shown in Fig. 1 and Table 1. As shown in Fig. 1, the logarithm of carbon concentration in the blood decreased linearly with time after injection except for high doses, 20 and 30 mg carbon. For the latter two doses the carbon concentration in the blood presented biphasic decrease with time. The first phase of the curve which lasted for the first ten to twenty minutes may be attributed to a small initial flocculation of carbon resulting from these high doses. In Table 1, the initial carbon concentration \( (C_0) \) is obtained by extrapolating these clearance curves to time 0. The blood volume \( (BV) \) calculated by dividing \( C_0 \) by dose was about 8 ml per 100 g body weight independent of the dose. The clearance of colloidal carbon was more rapid in the rats injected with low doses of carbon than in those with high doses.

**Table 1** Clearance of Colloidal Carbon from the Blood in the Rat after Single Intravenous Injection

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>No. of rats</th>
<th>Age (weeks)</th>
<th>K-value mean±s. d.</th>
<th>( C_0 ) mean±s. d.</th>
<th>( BV ) mean±s. d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>12</td>
<td>9-10</td>
<td>0.062±0.012</td>
<td>0.62±0.10</td>
<td>8.3±1.3</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>9</td>
<td>0.052±0.011</td>
<td>1.14±0.25</td>
<td>7.9±1.3</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>10</td>
<td>0.034±0.003</td>
<td>2.01±0.41</td>
<td>7.6±0.9</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>9</td>
<td>0.042±0.009*</td>
<td>2.86±0.31</td>
<td>7.0±0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.027±0.005*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>11</td>
<td>9</td>
<td>0.046±0.010</td>
<td>3.83±0.81</td>
<td>8.1±0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.021±0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* At doses of 20 and 30 mg, there were two phases of carbon clearance. See Fig. 1.
*** \( C_0 \) means initial concentration of carbon, mg carbon/ml blood.
**** BV means whole circulating blood volume per 100 g body weight.
Fig. 1 Concentration of colloidal carbon in the blood of the rats after single intravenous injection of various doses

Age and sex differences of clearance: The K-values were 0.060, 0.057, 0.034, 0.025, 0.021 and 0.022 for the rats, aged 3, 6, 10, 14, 36 and 68 weeks and injected with 15 mg colloidal carbon per 100 g body weight, respectively, showing that the clearance of colloidal carbon was more rapid in the young rats than in the old. Estimated BV and liver weight per 100 g body weight (W_L) decreased with the aging of the rat (Table 2). K-value, BV and W_L on the male and female rats aged 3, 10 and 14 weeks injected with 15 mg carbon per 100 g body weight are shown in Table 3. Sex difference of colloidal carbon clearance was noted significant in the rats aged 10 and 14 weeks, but not significant in those of 3 weeks old.

Clearance of 199Au-colloid: In order to determine the blood flow rate of the liver, clearance of 199Au-colloid was measured. K-values in the male rats of 7 and 17 weeks old, injected with 20 μCi of 199Au-colloid are showed in Table 4. K-values were 0.241 in the rats.
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of 7 weeks old and 0.169 in the rats of 17 weeks old. No difference was observed in K-values of clearance of the three dose levels of gold, i.e., 0.005, 0.010 and 0.015 mg per 100 g body weight.

Table 2  
Age Difference of Colloidal Carbon Clearance from the Blood of the Male Rats

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>No. of rats</th>
<th>K-value mean ± s. d.</th>
<th>Wt mean ± s. d.</th>
<th>BV mean ± s. d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6</td>
<td>0.060 ± 0.012*</td>
<td>4.66 ± 0.15*</td>
<td>9.1 ± 1.2*</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.057 ± 0.013*</td>
<td>4.37 ± 0.24*</td>
<td>8.5 ± 0.7*</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0.034 ± 0.003*</td>
<td>3.76 ± 0.68*</td>
<td>7.6 ± 0.9*</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>0.025 ± 0.007*</td>
<td>3.14 ± 0.32*</td>
<td>7.3 ± 1.0*</td>
</tr>
<tr>
<td>37</td>
<td>5</td>
<td>0.021 ± 0.004*</td>
<td>3.23 ± 0.08*</td>
<td>6.9 ± 0.7*</td>
</tr>
<tr>
<td>68</td>
<td>4</td>
<td>0.022 ± 0.003*</td>
<td>2.92 ± 0.23*</td>
<td>6.8 ± 0.9*</td>
</tr>
</tbody>
</table>

* Significant age difference was observed between a-b, a-c, a-d, b-c, b-d and c-d. (p < 0.05)
** Wt means liver weight per 100 g body weight.
*** BV means whole circulating blood volume per 100 g body weight.

Table 3  
Sex Difference of Colloidal Carbon Clearance from the Blood in the Rats

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Sex</th>
<th>No. of rats</th>
<th>K-value mean ± s. d.</th>
<th>Wt mean ± s. d.</th>
<th>BV mean ± s. d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>male</td>
<td>6</td>
<td>0.060 ± 0.012</td>
<td>4.66 ± 0.15</td>
<td>9.1 ± 1.2</td>
</tr>
<tr>
<td>female</td>
<td>7</td>
<td></td>
<td>0.059 ± 0.018</td>
<td>4.64 ± 0.42</td>
<td>9.2 ± 1.2</td>
</tr>
<tr>
<td>10</td>
<td>male</td>
<td>6</td>
<td>0.034 ± 0.003*</td>
<td>3.76 ± 0.68</td>
<td>7.6 ± 0.9*</td>
</tr>
<tr>
<td>female</td>
<td>10</td>
<td></td>
<td>0.041 ± 0.006*</td>
<td>4.10 ± 0.38</td>
<td>8.9 ± 1.2*</td>
</tr>
<tr>
<td>14</td>
<td>male</td>
<td>6</td>
<td>0.025 ± 0.007*</td>
<td>3.14 ± 0.32</td>
<td>7.3 ± 1.0</td>
</tr>
<tr>
<td>female</td>
<td>8</td>
<td></td>
<td>0.037 ± 0.008*</td>
<td>3.14 ± 0.37</td>
<td>7.8 ± 1.7</td>
</tr>
</tbody>
</table>

* Significant differences were observed between male and female (p < 0.05).
** Wt means liver weight per 100 g body weight.
*** BV means whole circulating blood volume per 100 g body weight.

Table 4  
Clearance of 198Au-colloid from the Blood of the Male Rats

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>No. of rats</th>
<th>Dose* (mg)</th>
<th>K-value mean ± s. d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>0.005</td>
<td>0.267 ± 0.038</td>
</tr>
<tr>
<td>4</td>
<td>0.010</td>
<td></td>
<td>0.241 ± 0.021**</td>
</tr>
<tr>
<td>5</td>
<td>0.015</td>
<td></td>
<td>0.260 ± 0.033</td>
</tr>
<tr>
<td>17</td>
<td>7</td>
<td>0.010</td>
<td>0.169 ± 0.041**</td>
</tr>
</tbody>
</table>

* dose: mg gold per 100 g body weight
** Significant age difference was observed between the rats of 7 weeks old injected with 0.010 mg gold and that of 17 weeks old injected with 0.010 mg gold (p < 0.05).
Age difference of extraction rate: Calculated extraction rate of colloidal carbon by the liver in the rats of 7 weeks old, injected with 5, 10 and 15 mg carbon per 100 g body weight is shown in Table 5. As is inferred from Table 2, the blood volume per 100 g body weight were about 8 ml in the rats of 7 weeks old and 7 ml in those of 17 weeks old. Accordingly, the rats of 17 weeks old were injected with 13 mg of colloidal carbon per 100 g body weight in order to make the initial concentration of carbon in the blood at the same level, i.e., 1.9 mg per ml in both groups. Extraction rate decreased as the dose of carbon increased, and was higher in the rats of 7 weeks old than in the 17 weeks old.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Dose (mg)</th>
<th>No. of rats</th>
<th>K-value mean ± s. d.</th>
<th>ER (%) mean ± s. d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>6</td>
<td>0.073 ± 0.010</td>
<td>46 ± 15.0*</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0.053 ± 0.008</td>
<td>27 ± 8.4b</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>0.045 ± 0.011</td>
<td>23 ± 6.5p</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>13</td>
<td>7</td>
<td>0.023 ± 0.005</td>
<td>11 ± 5.4c</td>
</tr>
</tbody>
</table>

* Significant difference was observed between a, b, c and a (p < 0.05).

**DISCUSSION**

The foreign particulate substances in the blood are mainly treated by the RES. In the previous studies (YAKUSIJI, 1965; SINGER et al., 1972) on the deposition pattern of particles using 198Au-colloid and colloidal carbon, it was shown that 80% or more of these colloidal carbon particles deposited in the liver, especially in the Kupffer cells of the liver.

When the particulate substances, such as colloidal carbon are injected intravenously and mixed instantly with the circulating blood and removed from the blood by RES at a constant rate, the rate of change in the concentration of the particles in the peripheral blood is represented by following equation (BIOZZI et al., 1953):

\[
\frac{dC}{dt} = -KC \quad \text{or} \quad C = C_0 e^{-kt} \quad \text{.........(1)}
\]

where C is the concentration of particulate substances and K is the ratio of the volume of the blood from which the particles are removed to the whole circulating blood volume. If we assume that the particulate substances are removed only by the liver, the following equation is derived from the equation (1) (DOBSON, 1953):

\[
HBF = BV \times \frac{K}{ER}
\]

where HBF is the liver blood flow, BV is the whole circulating blood volume and ER is the extraction rate of particles from the blood by the liver. This equation apparently indicates that HBF, BV and ER are the factors affecting K-value.

The clearance of colloidal carbon from the blood became slow as the injection dose of
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carbon increased (Fig. 1). It is proved by the present experiment, as is presented in Table 4, that this phenomenon was due to the decrease in ER. Other factors, i.e., HBF and BV, were not involved since they were not affected by the increase in the injection dose. The mechanism by which the increase in the injection dose causes the decrease in ER is not clear from this study, although it has been suggested that (1) saturation of RES-cells (NORMANN et al. 1965a, 1965b) or (2) shortage of some component of serum necessary for phagocytosis (JENKIN et al., 1961; BIOZZI et al., 1963; NORMANN et al., 1965a, 1965b; FILKINS et al., 1969) may account for this phenomenon.

The clearance rate of carbon from the blood as represented by K-value decreased not only with the increase in the injection dose but also with the aging of the rats as is shown in Table 2. In order to clarify the reason for this fact, clearance test of $^{198}\text{Au}$-colloid and measurement of extraction rate of carbon by the liver were carried out in the rats of 7 and 17 weeks old. As shown in Table 4, there are no variations in K-values for the dose range of 0.005 to 0.015 mg per 100 g body weight in the rats of 7 weeks old, suggesting that the clearance rate for the small amount of gold does not reflect the function and/or number of RES-cells, but the chance of gold particles meeting with RES-cells, i.e., liver blood flow as suggested by other authors (VETTER, 1954; VETTER et al., 1956; YAKUSIJI, 1965). Therefore, the observed difference between K-values of $^{198}\text{Au}$-colloid clearance in the rats of 7 weeks old and the rats of 17 weeks old may imply the decrease in the liver blood flow (HBF) as aging of rats. This hypothesis is supported indirectly by the fact that the liver weight per 100 g body weight decreased with the aging of rats, as is shown in Table 2. The value of ER for the rats of 7 weeks old, injected with 15 mg carbon is higher than that of 17 weeks old rats injected with 13 mg carbon, though both groups of rats had a same initial concentration of carbon. Since ER is considered to be a good indicator to the number and/or the function of RES-cells in the liver, the decrease in ER with aging of the rats indicates the decrease in the number and/or the function of RES-cells in the liver.

Dose, age and sex differences have been observed in the colloidal carbon clearance from the blood in the rat. The factors responsible for these differences are not only the function and/or number of RES-cells, but some physiological conditions such as the liver blood flow and the circulating blood volume. In actual practices, there may be cases where foreign particles enter the body through the inhalation or via the punctured wound but not via the intravenous route as used in this study. The movement of these particles are, therefore, more complex than that of carbon injected intravenously, and moreover, the toxicity of these particles may pose an additional complexity on their movement. In order to assess the systemic toxicity of particulate materials, it is essential to accumulate basic knowledge on the behavior of particles in the body by using several different types of simplified experimental systems and by using several kinds of biologically inert particles with uniform size.
SUMMARY

Wistar rats, aged 3 to 68 weeks, were injected with colloidal carbon solution. The peripheral blood samples collected at different times after injection were dissolved in 0.1% Na₂CO₃ solution and the amount of carbon in each blood sample was determined spectrophotometrically. Colloidal carbon of 5 to 30 mg per 100 g body weight was injected to the rats of 9 to 10 weeks old to examine the dose effect of colloidal carbon. The logarithm of carbon concentration in the blood decreased linearly with respect to the time after injection, except for high doses of 20 and 30 mg carbon. Therefore, the dose of 15 mg carbon per 100 g body weight was selected as a standard dose for the study of age and sex differences. K value, phagocytic index K, was calculated from the following equation:

\[ K = \frac{\log C_1 - \log C_2}{t_1 - t_2}, \]

where \( C_1 \) and \( C_2 \) are the concentrations of colloidal carbon at times \( t_1 \) and \( t_2 \). K-values were 0.060, 0.057, 0.034, 0.025, 0.021 and 0.022, for the rats aged 3, 6, 10, 14, 37 and 68 weeks, respectively, indicating that the clearance of colloidal carbon was more rapid in the young rats than in the old. It was demonstrated that whole circulating blood volume and liver weight per 100 g body weight decreased with the aging of rat. Sex difference of colloidal carbon clearance was significant in the rats of 10 and 14 weeks old, but not significant in the rats of 3 weeks old. Liver blood flow and extraction rate of colloidal carbon by the liver were measured in the rats of 7 and 17 weeks old in an attempt to identify the physiological factors involved in the sex and age differences. Both indicators were larger in the rats of 7 weeks old than in those of 17 weeks old. The results are indicating that age and sex differences of colloidal carbon clearance were affected not only by the function and/or the number of RES-cells but by such physiological factors as liver blood flow and whole blood volume.

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