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\[ ^{59}\text{Fe DISTRIBUTION AND ELIMINATION AFTER} \]
\[ \text{MELANIN ADMINISTRATION IN MICE} \]

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Melanin biopolymers demonstrate a high capacity to bind metal ions \textit{in vivo} and \textit{in vitro} [1 - 5]. It has been demonstrated that the model complexes of synthetic melanins as well as melanins isolated from natural sources with divalent and trivalent metal ions are characterized by relatively high stability and by two classes of independent binding sites [6, 7]. The interaction of synthetic DOPA melanin with iron(III) ions shows strong binding sites and weak binding sites represented by association constants \( K_1 = 1.65 \times 10^5 \text{M}^{-1} \) and \( K_2 = 5.22 \times 10^{-3} \text{M}^{-1} \) [7]. It has been also proved by the use of radiochemical methods that trivalent iron ions show the strongest affinity to DOPA melanin among seven metal ions (\( \text{Fe}^{3+}, \text{Cr}^{3+}, \text{Co}^{2+}, \text{Ni}^{2+}, \text{Zn}^{2+}, \text{Cd}^{2+}, \text{Mn}^{2+} \)) which have been tested [8]. The results obtained \textit{in vitro} were the base for studying the metal ions interaction with melanin in mice.

The aim of this work was to prove how far the administration of melanin into white mice (which do not contain melanin in their hair) and black mice (containing the melanin pigment) effects the \(^{59}\text{Fe} \) distribution and elimination outside the organism.

Melanin was obtained by oxidative polymerization of 3,4-dihydroxyphenylalanine (L-DOPA) in 0.067 M phosphate buffer at pH = 8.0,
according to the Binns method [9]. For injection, melanin was suspended in 0.9% NaCl containing 0.1% Tween 80, to the final concentration 10 mg melanin per 1 ml.

The experiments were carried out using male mice, strains Balb c and C-57 Bl. of 25.0 ± 3.0 g weight. Control as well as experimental groups of mice were injected intraperitoneally with 0.5 ml of $^{59}$Fe solution (specific activity 31.1 GBq/g Fe, dose 350 kBq/kg body weight). In the same way 0.5 ml aliquots of melanin suspension were administered to the experimental groups in the dose 200 mg/kg body weight i.e. 5.0 mg/mouse.

Radioactivity measurements were performed by the use of gamma scintillation spectrometer (Polon, Poland).

The mice were decapitated after 21 days of experiment. Blood was drawn and the following organs were isolated and examined: liver, heart, lungs, kidneys, spleen and pieces of skin. The organs were weighed, subjected to radioactivity measurements and the results were expressed as radioactivity in c.p.s./gram of mouse or tissue and in case of blood in c.p.s./ml. For statistic evaluation t-Student's test was used. Histochemical examinations were carried out by staining of tissue slides by the use of hematoxyline.

$^{59}$Fe radioactivities of the experimental mice in the time course of the experiment are presented in Figure 1. Generally, the higher $^{59}$Fe elimination in black C-57 Bl. mice was demonstrated as compared with white Balb c mice. At the same time no statistically significant differences between $^{59}$Fe elimination in both the melanin treated and control groups were found.

Distribution of $^{59}$Fe in mice organs is shown in Table 1. The highest radioisotope concentration was found in blood, spleen, heart and lungs and the lowest one — in the liver. To compare the $^{59}$Fe distribution in mice organs of melanin treated and control groups, the radioactivity found in each of the examined organs was calculated as percent of radioactivity with the reference to organs of control groups. Relative $^{59}$Fe radioactivities of mice organs are presented in Figure 2 (for white mice of Balb c strain) and in Figure 3 (for black C-57 Bl. mice).

Higher values of relative radioactivity in mice organs were found in C-57 Bl. as compared with Balb c mice. The largest differences in $^{59}$Fe concentration were observed in kidneys, where the relative radioactivity in C-57 Bl. mice was 132.4% and in the Balb c only 86.7%. Histopathological evaluation of slides obtained from mice organs shows that i.p. injection of exogenous melanin to mice causes the accumulation of the melanin in liver and spleen of both C-57 Bl. and Balb c strains in the form of melanin
Fig. 1. Retention of $^{59}$Fe in mice
Fig. 2. Relative $^{59}$Fe radioactivity in Balb c mice organ

Fig. 3. Relative $^{59}$Fe radioactivity in C-57 Bl. mice organ
Table 1

$^{59}$Fe distribution in mice organs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Radioactivity (c.p.s./g)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>C-57 B1</td>
<td>Balb c</td>
</tr>
<tr>
<td></td>
<td>Melanin</td>
<td>Control</td>
<td>Melanin</td>
</tr>
<tr>
<td>Mouse</td>
<td>112 ± 11</td>
<td>112 ± 14</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>Skin</td>
<td>112 ± 10</td>
<td>102 ± 11</td>
<td>161 ± 5</td>
</tr>
<tr>
<td>Liver</td>
<td>156 ± 12</td>
<td>174 ± 19</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>Heart</td>
<td>204 ± 17</td>
<td>234 ± 21</td>
<td>609 ± 27</td>
</tr>
<tr>
<td>Lungs</td>
<td>215 ± 19</td>
<td>216 ± 19</td>
<td>598 ± 28</td>
</tr>
<tr>
<td>Kidneys</td>
<td>188 ± 9</td>
<td>142 ± 13</td>
<td>242 ± 18</td>
</tr>
<tr>
<td>Spleen</td>
<td>278 ± 91</td>
<td>277 ± 23</td>
<td>603 ± 26</td>
</tr>
<tr>
<td>Blood</td>
<td>743 ± 35</td>
<td>741 ± 29</td>
<td>319 ± 14</td>
</tr>
</tbody>
</table>

capsules. The presence of melanin in the hair and hair follicles was ascertained only in C-57 Bl. mice after administration of melanin but was not found in Balb c mice.

The higher elimination of $^{59}$Fe from black mice as compared with white mice when not treated with melanin, the localization of the administered melanin in the form of capsules on the liver and spleen surfaces in both the black and the white mice, and the effect of exogenous melanin on the concentration of $^{59}$Fe in various specific tissues suggest that the applied melanin to $^{59}$Fe contaminated mice may be used for isotopic decontamination.

REFERENCES