The occurrence of trivalent chromium in the aqueous and lens of rats*

Tibor G. Farkas and Jelka Pluščec

It has been demonstrated that trivalent chromium reaches the aqueous and lens of normal rats. Trivalent chromium accumulates in the isolated lens in trace quantity and the metal localizes in and about the capsule.

It has been reported\(^1\), \(^2\) that trivalent chromium plays an important role in insulin action. We have shown\(^3\) that, in the presence of trivalent chromium, insulin stimulated the glucose utilization of isolated rat lenses. This finding suggested the possibility that a variation in dietary chromium might be responsible for the repeated inconsistency in the insulin effect observed on the glucose utilization by the isolated lens. To evaluate further the role of chromium in lens metabolism, it was thus necessary to know the amount of trivalent chromium reaching the lens within the eye. We have investigated the trivalent chromium content of the aqueous humor and the lens, since no data were available concerning ocular chromium concentrations.

Method

Male Sprague-Dawley rats, weighing 120 to 150 grams, were used in all experiments. The animals were kept on Purina Rat Chow and distilled, deionized water.

To study the trivalent chromium content of the aqueous, the animals received by intraperitoneal injection 0.38 \(\mu\) moles of trivalent chromium for three days. Trivalent chromium-51\(^*\) (in \(\text{Cr}^{51}\text{Cl}_3\) form) was injected intravenously at the start of the experiment. The animals were anesthetized with intraperitoneal sodium pentobarbital (6 mg. per 100 Gm.).\(^f\) At various time intervals after \(\text{Cr}^{51}\text{Cl}_3\) injection, the eyes were rinsed with Tyrode's solution containing nonradioactive trivalent chromium and 10 \(\mu\)l of aqueous humor was removed from each anterior chamber. The aqueous humor was added to 5.0 ml of distilled water and counted in duplicate in the gamma well of a Packard\(^t\) scintillation counter. One animal was used at each time period and samples of aqueous removed from both eyes permitted duplicate counting.

To study the trivalent chromium content of the lens capsule the animals were pretreated with chromium as described above. Thirty minutes after the injection of trivalent chromium-51 (in \(\text{Cr}^{51}\text{Cl}_3\) form) the aqueous was removed as described above. After the removal of the aqueous the lenses were removed and rinsed with cold (4° C.) solution containing nonradioactive trivalent chromium. The lenses were blotted dry and the capsules were removed under the dissecting microscope, combined, and weighed. The capsules were then placed into distilled water and counted in the gamma well of the scintillation

\(\*\)Volk Radiochemical Co., Skokie, Ill.
\(\f\)Diamond Laboratories, Des Moines, Iowa.
\(\t\)Packard Instrument Co., Inc., La Grange, Ill.
counter. Blood was withdrawn from these animals by a heparinized syringe. The cellular components were separated by centrifugation and the clear plasma was counted in the open well of the scintillation counter.

To study the interaction of trivalent chromium and the isolated lens, the animals were decapitated, and the lenses were removed and weighed. The lenses were incubated for 30 min. at 37° C. in Tyrode's solution containing 1.0 mg. per milliliter of glucose and variable concentration of trivalent chromium (in CrCl₃ form). Following incubation, the lenses were removed from the medium, rinsed with nonradioactive chromium solution (1 μg per milliliter) at 4° C., blotted dry, and counted in the gamma well of the scintillation counter.

In separate experiments, following incubation of isolated lenses as described above, the lenses were rinsed with nonradioactive chromium solution (1 μg per milliliter) at 4° C. The capsules were removed under a dissecting microscope. The capsules were weighed and counted individually, as were the remaining lenses.

### Table I. Change in trivalent chromium-51 content of aqueous with varying amount of trivalent chromium-51 administration

<table>
<thead>
<tr>
<th>Specific activity of injected ⁵¹Cr³⁺⁺⁺</th>
<th>⁵¹Cr³⁺⁺⁺ injected per 100 Gm. rat (μ moles)</th>
<th>Time (min.)</th>
<th>⁵¹Cr³⁺⁺⁺ per ml. of aqueous (μ moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mc. per mg.)</td>
<td>(μ moles)</td>
<td>1</td>
<td>0.26 × 10⁻⁴</td>
</tr>
<tr>
<td>169.90</td>
<td>4.66 × 10⁻²</td>
<td>2</td>
<td>0.43 × 10⁻⁴</td>
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<td></td>
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<td>3</td>
<td>0.66 × 10⁻⁴</td>
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<td>6</td>
<td>0.86 × 10⁻⁴</td>
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<td>10</td>
<td>0.41 × 10⁻⁴</td>
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<td>12</td>
<td>0.17 × 10⁻⁴</td>
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<td>30</td>
<td>0.39 × 10⁻⁴</td>
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<td></td>
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<td>2.5</td>
<td>0.54 × 10⁻⁴</td>
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<td>5</td>
<td>1.27 × 10⁻⁴</td>
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<td>10</td>
<td>0.50 × 10⁻⁴</td>
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<td>27</td>
<td>0.54 × 10⁻⁴</td>
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<td>3</td>
<td>0.25 × 10⁻⁴</td>
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<td>9</td>
<td>0.69 × 10⁻⁴</td>
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<td>11</td>
<td>0.72 × 10⁻⁴</td>
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<td>13</td>
<td>0.84 × 10⁻⁴</td>
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<td>15</td>
<td>0.72 × 10⁻⁴</td>
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<td></td>
<td></td>
<td>90</td>
<td>0.65 × 10⁻⁴</td>
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<td></td>
<td>120</td>
<td>0.28 × 10⁻⁴</td>
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<td>3</td>
<td>0.73 × 10⁻⁴</td>
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<td>7</td>
<td>1.02 × 10⁻⁴</td>
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<td>9</td>
<td>5.10 × 10⁻⁴</td>
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<td>1.37 × 10⁻⁴</td>
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<td>13</td>
<td>0.95 × 10⁻⁴</td>
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</table>
Table II. Trivalent chromium-51 content of plasma, aqueous, and lens capsules of \(^{51}\text{Cr}^{+++}\)-injected animals

<table>
<thead>
<tr>
<th>Specific activity of injected (^{51}\text{Cr}^{+++})</th>
<th>(^{51}\text{Cr}^{+++}) injected per 100 Gm. rat ((\mu) moles)</th>
<th>(^{51}\text{Cr}^{+++}) per ml. of plasma ((\mu) moles)</th>
<th>(^{51}\text{Cr}^{+++}) per ml. of aqueous ((\mu) moles)</th>
<th>(^{51}\text{Cr}^{+++}) per mg. of capsule (wet weight) ((\mu) moles)</th>
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</thead>
<tbody>
<tr>
<td>293</td>
<td>9.9 (\times 10^{-2})</td>
<td>9.80 (\times 10^{-2})</td>
<td>0.69 (\times 10^{-1})</td>
<td>0.12 (\times 10^{-2})</td>
</tr>
</tbody>
</table>

Samples removed 30 min. after \(^{51}\text{Cr}^{+++}\) injection.

Table III. Trivalent chromium-51 content of isolated rat lenses

<table>
<thead>
<tr>
<th>Specific activity of (^{51}\text{Cr}^{+++}) used (mc. per mg.)</th>
<th>(^{51}\text{Cr}^{+++}) content of media ((\mu) moles per ml.)</th>
<th>(^{51}\text{Cr}^{+++}) per Gm. of wet weight ((\mu) moles)</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.95</td>
<td>2.14 (\times 10^{-4})</td>
<td>1.35 (\times 10^{-4}) (12)</td>
<td>(\pm 1.12 \times 10^{-6})</td>
</tr>
<tr>
<td>45.95</td>
<td>1.69 (\times 10^{-4})</td>
<td>1.19 (\times 10^{-4}) (12)</td>
<td>(\pm 2.02 \times 10^{-6})</td>
</tr>
<tr>
<td>169.90</td>
<td>0.49 (\times 10^{-4})</td>
<td>1.15 (\times 10^{-4}) (8)</td>
<td>(\pm 1.29 \times 10^{-6})</td>
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</tbody>
</table>

Incubation medium, Tyrode's solution containing 1 mg. of glucose per ml. Volume of incubation medium, 5 ml. Temperature, 37°C. Time of incubation, 30 min.

*Number in parentheses is number of lenses.

Table IV. Trivalent chromium-51 content of lens capsule, cortex, and nucleus content

<table>
<thead>
<tr>
<th>Specific activity of injected (^{51}\text{Cr}^{+++}) (mc. per mg.)</th>
<th>(^{51}\text{Cr}^{+++}) content of media ((\mu) moles per ml.)</th>
<th>(^{51}\text{Cr}^{+++}) in capsule ((\mu) moles per Gm. of wet weight) Standard error</th>
<th>(^{51}\text{Cr}^{+++}) in cortex and nucleus ((\mu) moles per Gm. of wet weight) Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.95</td>
<td>2.14 (\times 10^{-4})</td>
<td>1.36 (\times 10^{-3}) (9)</td>
<td>(\pm 1.29 \times 10^{-3}) (9)</td>
</tr>
</tbody>
</table>

Incubation medium, Tyrode's solution containing 1 mg. of glucose per ml. Volume of incubation medium, 5 ml. Time of incubation, 30 min. Temperature, 37°C.

*Number in parentheses is number of capsules and cortices and nucleus.

the incubation medium is not significantly influenced by the chromium content of the medium.

All trivalent chromium which accumulates in the lens can be found in the lens capsule (Table IV). The cortex and nucleus contain only minute amounts of trivalent chromium.

The radioautograph (Fig. 1) indicates that chromium accumulates in the proximity of the lens capsule. There seems to be no penetration of the trivalent chromium into the central portion of the lens. This is in good agreement with the analysis of capsules and lens substance (Table IV).

Discussion

We have suggested previously that the stimulatory effect insulin exerts on the glucose utilization of the rat lens is dependent on trivalent chromium. This hypothesis necessitates the entry of chromium into the eye if the metal ion is to be active at the ocular level. These results demonstrate that trivalent chromium passes through the blood-aqueous barrier and can be detected in trace amounts in the aqueous. We have shown that trivalent chromium interacts with the lens and the lenticular concentration of the metal ions is not a function of trivalent chromium concentration in the medium.

The capsule contains almost all the trivalent chromium in the lens. One would expect this observation if the role of the metal is to enhance the attachment of insulin to surface membrane as proposed by Christina and associates.
Even though these findings do not elucidate further the role of chromium and insulin on lens metabolism, the results demonstrate that the metal ion could participate in trace quantities at the lenticular level in the glucose utilization of the lens.

REFERENCES