THE ANEMIA OF INFECTION

XI. THE EFFECT OF TURPENTINE AND COBALT ON THE ABSORPTION OF IRON BY THE RAT*

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In a previous report (1) from this laboratory it was demonstrated that by the simultaneous administration of cobalt the anemia associated with inflammation, as produced by the injection of turpentine, was not only prevented from developing but polycythemia was produced instead. In Paper X of this series (2) it was demonstrated that the absorption of iron from the gastrointestinal tract was decreased in rats in which a bacterial infection was produced. The purpose of this paper is to determine whether sterile (turpentine) abscesses have a similar effect on iron absorption, and, if so, whether the simultaneous administration of cobalt can reverse this effect. Theoretically, the iron incorporated into the newly synthesized hemoglobin of cobalt-treated, turpentine-injected, polycythemic rats could come from either tissue stores or the gastrointestinal tract or from both sources.

EXPERIMENTAL

Animals—A total of 57 weanling male Sprague-Dawley rats was used in this study. The animals were fed the purified diet, without added iron, described in Paper X (2). All animals received 0.5 ml. of iron solution, prepared as described previously (2), containing 2 mg. of total iron as FeCl₂·4H₂O and 622 countable counts of radioactive iron per minute by stomach tube 24, 48, and 96 hours after each turpentine injection. Each rat received a total of thirteen such iron feedings, representing a total of 8086 countable counts per minute of radioactivity. 5 days were allowed to elapse between the last iron feeding and sacrificing the animals. All animals were killed by ether anesthesia 32 days after the first turpentine injection.

Nine rats were used as controls and were given neither turpentine nor cobalt. In eighteen rats 0.25 ml. of rectified oil of turpentine (Rexall) was injected each week intramuscularly into the hind leg. Twelve rats

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were given 0.125 mg. of cobalt daily as cobaltous chloride in an aqueous solution, injected intramuscularly. Eighteen rats received both turpentine and cobalt in the amounts stated above. Only fourteen of the thirty-six rats receiving turpentine survived.

Methods—The wet digestion of the whole rat, the dry ashing of the wet digest, and the separation and measurement of the radioactive iron were carried out as described in Paper X (2). Hemoglobin determinations were made on tail blood at weekly intervals by the oxyhemoglobin method and with the Evelyn photoelectric colorimeter.

### Table I

**Effect of Turpentine, Cobalt, and Cobalt Plus Turpentine**

The results are expressed in counts per minute.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Controls</th>
<th>Turpentine-treated</th>
<th>Cobalt-treated</th>
<th>Cobalt + turpentine-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per rat</td>
<td>Per 100 gm.</td>
<td>Per rat</td>
<td>Per 100 gm.</td>
</tr>
<tr>
<td>1</td>
<td>972</td>
<td>954</td>
<td>832</td>
<td>650</td>
</tr>
<tr>
<td>2</td>
<td>1080</td>
<td>940</td>
<td>716</td>
<td>619</td>
</tr>
<tr>
<td>3</td>
<td>856</td>
<td>586</td>
<td>766</td>
<td>556</td>
</tr>
<tr>
<td>4</td>
<td>1104</td>
<td>758</td>
<td>640</td>
<td>478</td>
</tr>
<tr>
<td>5</td>
<td>960</td>
<td>667</td>
<td>1052</td>
<td>756</td>
</tr>
<tr>
<td>6</td>
<td>1272</td>
<td>889</td>
<td>988</td>
<td>667</td>
</tr>
<tr>
<td>7</td>
<td>816</td>
<td>597</td>
<td>728</td>
<td>509</td>
</tr>
<tr>
<td>8</td>
<td>976</td>
<td>646</td>
<td>988</td>
<td>772</td>
</tr>
<tr>
<td>9</td>
<td>1056</td>
<td>714</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1010</td>
<td>752</td>
<td>822</td>
<td>591</td>
</tr>
<tr>
<td>Weight, gm.</td>
<td>136 ± 17</td>
<td>139 ± 7</td>
<td>143 ± 9</td>
<td>132 ± 6</td>
</tr>
<tr>
<td>Hb, gm. per 100 ml.</td>
<td>16.4 ± 0.66</td>
<td>14.4 ± 2.67</td>
<td>20.3 ± 1.01</td>
<td>18.6 ± 1.76</td>
</tr>
</tbody>
</table>

**Results**

The results are presented in Table I. The values for the body weight and final hemoglobin represent the averages for the groups; those for the total body radioactive iron in counts per minute are given in detail for each rat.

The rats injected with turpentine developed significant anemia in the 32 day period, whereas not only the cobalt-treated rats but also those receiving turpentine plus cobalt developed a significant degree of polycythemia in that time as judged by the hemoglobin values. Accompanying the decrease in hemoglobin production shown by the turpentine-
injected rats there was a decrease in iron absorption as demonstrated by the low total radioactive iron content of the body compared to the controls. The difference in counts per minute between the control and turpentine-injected groups is significant by Fisher's t test (3) \((t = 2.48)\). This is true whether the counts are figured per rat or per 100 gm. of rat.

The rats given cobalt and those receiving cobalt plus turpentine showed a somewhat greater total body content of radioactive iron than the controls, but the differences are not significant. However, the difference between the turpentine and turpentine plus cobalt groups is highly significant \((t = 6.27)\).

**DISCUSSION**

These experiments confirm our previous observation (1) that anemia does not develop following the production of turpentine abscesses if cobalt is injected. They are also consistent with the observation (4–6) that anemia associated with infection in human subjects can be influenced by the administration of cobalt. It is evident from the data, furthermore, that sterile abscesses, like bacterial abscesses and infections (2), are associated with a decrease in the absorption of iron from the gastrointestinal tract. The administration of cobalt to animals with sterile abscesses not only abolished the anemia-producing effects of turpentine and produced a polycythemia, but it also increased the absorption of iron from the gastrointestinal tract to within the normal range. In the cobalt-treated animals, most of the iron for the synthesis of the additional hemoglobin came from the gastrointestinal tract, even though the absorption of iron was not increased beyond normal in this group. This is in keeping with, and helps to explain, the observation that cobalt does not produce polycythemia when the diet is deficient in iron (7).

From this it must not be concluded that the principal action of cobalt is on the absorption of iron and that polycythemia is a result of this action. If this were true, the administration of iron parenterally should result in polycythemia, and such is not the case. It is more logical to presume that the action of cobalt is exerted in some other way and that, as a result of this, more iron is absorbed. Orten and Bucciero (8) have suggested that cobalt may produce polycythemia by binding sulfhydryl or perhaps other groups active in cellular respiration, thus leading to a simulated cellular anoxia and, in turn, to a compensatory polycythemia. If this is true, then, just as in hemorrhagic anemia, the rate of hemoglobin synthesis is accelerated, the need of the body for iron is increased, and the absorption of iron is enhanced.

Although cobalt completely counteracted the anemia-producing effects of turpentine and restored the amount of iron absorbed to normal, this
substance, in the presence of a turpentine abscess did not produce the same degree of polycythemia as in the animals treated with cobalt alone. This was true, even though the absorption of iron in the animals treated with cobalt and turpentine was approximately equal to that of the animals treated with cobalt alone. Thus cobalt must have produced a greater increase in tissue iron in animals injected with turpentine than in animals treated with cobalt alone.

SUMMARY

The influence of turpentine-induced abscesses and of cobalt administration on the absorption of iron has been investigated in rats by determining the total amount of radioactive iron retained in the body during the period of treatment.

Turpentine was found to decrease the absorption of iron, and this effect could be reversed by the administration of cobalt.

Cobalt administration did not significantly affect the absorption of iron in normal rats.

BIBLIOGRAPHY

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