STUDIES ON THE MECHANISM OF COBALT POLYCYTHEMIA

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Although it is perhaps generally recognized that the mechanism of cobalt polycythemia is not completely understood, the work most frequently cited is that of Barron and Barron (1) to the effect that a small amount of cobalt inhibits the respiration, in vitro, of various tissues but notably reticulocytes and bone marrow. These authors propose that cobalt polycythemia is due "to inhibition by cobalt of the respiratory function of immature red cells. Once these cells have lost their ability to respire, they are thrown into the circulation as mature non-respiring cells, being replaced in the bone marrow by new cells". Barron and Barron's own data (their table 1) show, however, that the reticulocytes and erythroblasts that appear in the bloodstream in cobalt polycythemia are actively-respiring cells. It has been shown from other studies, however, that cobalt produces increased erythropoietic activity with erythroid metaplasia in the marrow (2,3,4) and a reticulocytosis and polycythemia in the circulating blood (5,6). It is rather difficult to understand how this increased activity of the marrow could occur in the face of impaired respiratory activity, particularly since Warren (7) found that erythroid marrow cells are characterized metabolically by active respiratory and low glycolytic activity. If respiration is impaired, how could the cells undergo more rapid maturation and growth than normally; would they obtain the required energy from increased glycolytic activity? It was largely with these questions in mind that the present study was undertaken but we have also investigated several other possible modes of action of cobalt. Unfortunately, the results in each instance are essentially negative, but it is our hope that the findings to be presented will clarify the work in this field.

A. Marrow Respiration and Glycolysis in Cobalt Polycythemia. 1. The effect on marrow respiration of adding cobalt in vitro. These experiments are essentially a repetition of those of Barron and Barron (1). Solutions of CoSO₄ have been tipped onto slices of rabbit bone marrow while the respiration was being measured in the Warburg apparatus. Fifteen experiments have been carried out, with the final concentration of cobalt in the vessels varying from 10⁻⁴ to 10⁻² molar. Various media have been employed,—neutralized serum (8), saline and Ringer solution, all with and without added phosphate and glucose. Marrows from normal and from cobalt polycythemic animals have been employed, and in other experiments suspensions of rabbit red cells rich in reticulocytes and suspensions of nucleated red cells of ducks have been used. In no instance was an appreciable effect of cobalt observed during the 3-hour experimental period following its addition unless the concentration was as high as 0.01 M when depressions of

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themia, seven experiments were performed in which the respiration of normal
marrows was measured both in normal serum and in that from cobalt-polycythemic animals. Respiration in the serum of the polycythemic animals averaged only 1 per cent less than in normal serum, with a P.E. of ± 3.8 per cent. In nine experiments, the reverse determination was carried out, respiration of the marrow from cobalt-polycythemic animals being measured in serum from both normal and polycythemic animals. The rate of respiration averaged 3 per cent lower in the normal serum, with a P.E. of ± 4.6 per cent. Clearly, no significant effect of the sera on either normal or polycythemic marrows could be established.

B. Possible Neural Mode of Action of Cobalt. In a series of papers (12-16) Davis has demonstrated that choline and certain other vasodilator drugs depress or prevent the polycythemia following cobalt administration, whereas vasoconstrictor drugs can induce polycythemia. The action of vasodilator drugs in inhibiting cobalt polycythemia is interpreted by Davis in terms of the Barron-and-Barron theory, i.e., the inhibitory action of cobalt on the respiratory activity of the erythroid marrow cells is counteracted by the increased oxygen supply to the marrow resulting from the vasodilatation. Since we cannot confirm the Barron and Barron theory of impaired respiration of the marrow cells, an alternative explanation of the antagonistic action of cobalt and the vasodilator drugs suggested itself, namely, that cobalt might act by a local neural mechanism (vasoconstriction?) that is directly counteracted by choline. If this were true, denervation of the marrow should prevent the effect of cobalt.

This possibility was investigated by inducing cobalt polycythemia in a series of rabbits in which one hind limb was denervated, and making morphological examination of the tibial marrow of the normal and denervated limbs. Eight animals were used in the experiments. The operation consisted of section under local anesthesia of the right femoral nerve as it emerges beneath the inguinal ligament, and of the right sciatic nerve in the upper third of the thigh. At least a centimeter of nerve was removed distal to these points. This procedure destroys both the somatic and autonomic innervation of the right hind leg in most of the thigh; below the knee, the denervation is complete. This was verified by physiological examination after the animals had recovered from the operation, and later when they were sacrificed after production of cobalt polycythemia. Complete sensory and motor paralysis was always observed below the level of the knee, and in the later examinations there was marked atrophy of the thigh and leg muscles. The innervation of the vascular supply of the tibial marrow must consequently have been interrupted, for it has been shown that the sympathetic innervation of vessels in the extremities is via the peripheral nerves and that the fibers do not traverse the vessels for more than a short distance (17).

The data on these animals are summarized in table 2. After the operation, cobalt sulfate was administered either orally or subcutaneously in doses of 5 or 7 mgm. cobalt per day together with 3 mgm. of MnCl₂ (18) for periods varying from 19 to 81 days. Usually about 3 weeks were required for the polycythemia to reach its maximum degree, after which it was maintained. The original and
final levels of the red blood cell count and hemoglobin determinations are shown in the table. No difference was noted either in the rate of development or in the degree of polycythemia in the operated animals compared with 7 others in which no operation was performed. When the animals were sacrificed, the gross appearance of the femoral and tibial marrow of the normal and denervated limbs was identical. All the marrows appeared to be hyperplastic but to a variable degree in different animals. This was confirmed by examination of sections prepared from corresponding areas of the tibial marrow on the two sides. The degree of hyperplasia was graded on an arbitrary scale from + to +++. These gradings are shown in table 2. It is clear that the same extent of erythroid hyperplasia was present in the control and denervated limbs.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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</table>

**Blood and bone marrow responses following administration of cobalt to animals with a denervated hind limb**

<table>
<thead>
<tr>
<th>EXP. NO.</th>
<th>COBALT DOSAGE</th>
<th>DURATION OF EXP.</th>
<th>RED BLOOD CELL COUNT</th>
<th>HEMOGLOBIN</th>
<th>PER CENT ERYTHROID MARROW CELLS</th>
<th>MARROW HYPERPLASIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mgm/day</td>
<td>days</td>
<td>Begin.</td>
<td>End</td>
<td>Begin.</td>
<td>End</td>
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</tr>
<tr>
<td>6</td>
<td>5</td>
<td>41</td>
<td>5.3</td>
<td>6.8</td>
<td>12.3</td>
<td>15.6</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>61</td>
<td>5.3</td>
<td>8.0</td>
<td>9.0</td>
<td>13.5</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>63</td>
<td>6.2</td>
<td>7.5</td>
<td>7.9</td>
<td>12.5</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>67</td>
<td>5.6</td>
<td>7.1</td>
<td>5.0</td>
<td>8.0</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>29</td>
<td>4.3</td>
<td>7.8</td>
<td>10.7</td>
<td>16.3</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>81</td>
<td>4.7</td>
<td>7.4</td>
<td>10.4</td>
<td>14.9</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>19</td>
<td>4.2</td>
<td>7.6</td>
<td>9.5</td>
<td>13.7</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>21</td>
<td>5.4</td>
<td>8.0</td>
<td>10.9</td>
<td>13.8</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>5.1</td>
<td>7.5</td>
<td>10.7</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Finally, differential bone marrow counts were made on smears of the tibial marrow of the control and denervated limbs. The proportion of erythroid cells in the marrow of normal rabbits of the same strain is slightly less than 50 per cent (7). In the present series, as shown in table 2, the average proportion of erythroid cells was 60 per cent, both in the control and denervated marrows. This would hardly be a significant increase were it not for the hyperplasia which occurred concomitantly; when these two changes are considered together, it is clear that a definite increase occurred in both the mass and proportion of erythroid cells and that these changes were independent of whether or not the marrow had an intact innervation. We conclude that the morphological response of the bone marrow to cobalt is independent of an intact peripheral innervation.3

C. Histological Changes in Marrow Blood Vessels. Reznikoff, Foot and

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3 It is also worthy of note that denervation does not affect the cellular composition of normal marrow or alter the marrow response to low oxygen tension (19).
Bethea (20) have reported striking morphological changes in the vascular system of the bone marrow in cases of polycythemia vera. These include thickening and sclerosis of the vascular walls and the occurrence of thromboses. We have examined the marrows of our cobalt polycythemic animals for these vascular changes, using the same technique described by Reznikoff et al. No such changes were found and in fact there was no evidence of any morphological changes in the vessels. Dorrance and his collaborators (4) have also reported recent pathologic studies in which no vascular changes were noted.

Discussion. In the foregoing sections, we have been unable to confirm the thesis that cobalt acts by impairing the respiratory activity of the bone marrow and have shown that its effects are independent of the peripheral innervation of the marrow and do not involve morphological changes in the marrow vessels. How it acts is certainly not elucidated, but in the spirit of stimulating further investigation, two possibilities might be entertained. 1. It could act on the liver, possibly enhancing the formation there of metabolic precursors requisite for red cell production. That choline, its antagonist, acts there is well known. 2. The above hepatic mechanism might be set off by cobalt acting on a central neural mechanism. Incidentally, a hypothetical case may be made out for oxygen lack acting by way of a central neural mechanism also, but further speculation along these lines is obviously unwarranted.

SUMMARY AND CONCLUSIONS

1. The observation of Barron and Barron that the respiration of bone marrow and reticulocytes in vitro is impaired by small amounts of cobalt cannot be confirmed.

2. The respiration and glycolysis of the marrow of cobalt-polycythemic animals is slightly lower than that which would be predicted from previous studies, but the difference is so slight as to be of very doubtful significance.

3. The in vitro respiration of both normal and polycythemic marrow is not altered by substituting polycythemic for normal serum.

4. The erythroid hyperplasia of bone marrow in cobalt-polycythemic animals is independent of whether or not the marrow has an intact peripheral innervation.

5. No morphological changes were observed in the blood vessels of marrow from cobalt-polycythemic animals.

6. In our opinion, the mechanism of the action of cobalt in inducing polycythemia is unexplained; several possibilities are suggested for further study.

REFERENCES

(2) Mascherpa, P. Haematologia 10: 361, 1929.
(12) Davis, J. E. This Journal 127: 322, 1939.
(13) Davis, J. E. This Journal 129: 140, 1940.
(15) Davis, J. E. This Journal 134: 219, 1941.
(16) Davis, J. E. This Journal 137: 94, 1942.
(18) Kleinberg, W. This Journal 108: 545, 1934.