Experimental Red Cell Chimerism in the Heterozygote (Wv'w) of the W-Series Mutants in the House Mouse

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Mice of the genotype Wv'Wv have an inherited macrocytic anaemia. Bernstein and Russell (1959) found that after the administration of isologous haematopoietic tissue these anaemic animals would permanently gain a normal blood picture. Seller and Polani (1966) then showed that the same result could be achieved using homologous foetal liver cells. The presence of donor type haemoglobin was detected in all treated Wv'Wv subjects up to one year after the transplantation, providing evidence that donor cells had become permanently implanted in the body of the host and were continuing to function according to their own genotype (Seller, 1966). Heterozygote animals of the W-series, of the genotype Wv'w, are also anaemic, but are less severely affected than the Wv'Wv as far as red cell numbers are concerned, and there is only slight macrocytosis (Grüneberg, 1942; Russell and Fondal, 1951).

The present work describes how Wv'w animals were treated in a similar manner to the homozygotes Wv'Wv, and given homologous erythropoietic tissue. It was found that they, too, gained a normal peripheral blood picture. The presence of donor type haemoglobin suggests that they are erythropoietic chimeras.

Material and Method

Details of the transplantation technique have been described previously (Seller and Polani, 1966). Animals of the Wv'w genotype were injected intravenously, within a few hours of birth, with a liver cell suspension (approximately 6 - 10 x 10⁶ cells) prepared from 15-day-old foetuses of haematologically normal mice from either of two unrelated strains. The donor mice were a closed colony A strain, and a pure line CBA strain.

When the treated mice were adult, the peripheral red blood picture was examined. Blood was taken from the warmed tail of the animal and haematocrit values estimated using the Hawksley microhaematocrit centri-fuge, and red blood cell counts made by conventional haemocytometer methods using Hayem's fluid as diluent.

Electrophoresis of the haemoglobins was performed using cellulose acetate paper as the supporting medium and a barbitone buffer at pH 8.6, ionic strength 0.05. It was run for 3 hours at room temperature at 5 volts/cm. length. Afterwards the papers were stained with a 0.2% solution of Ponceau S in 3% trichloracetic acid.

Five series of mice were studied haematologically: the donor A, and the donor CBA (these groups have been mentioned in previous papers — Seller and Polani, 1966; Seller, 1966), the Wv'w animals treated with A foetal liver cells, and the Wv'w—CBA treated animals, and a control set of Wv'w individuals which had received no treatment, but were matched for age with the test animals.

Results

Wv'w animals, aged approximately 18 months, of the colony held by this laboratory were found to have a mean red blood count of 8.84 x 10⁶ cells/mm.³ compared with the normal (approximately 11.5 x 10⁶/mm.³), and a mean haematocrit value of 38.2%. There was evidence of a slight macrocytosis, the mean corpuscular volume being 43.3 μm².

Six of the 10 Wv'w animals injected with A foetal liver cells, and six of seven of the CBA-treated Wv'w mice showed an altered peripheral blood picture using the criteria of haematocrit value, mean corpuscular volume, and red cell counts, from the anaemic level to one typical of the normal A and CBA mice (Table ). This appears to be a permanent change, for it is still demonstrable in animals which are 18 months of age.

Electrophoresis of the haemoglobin of Wv'w, A, and CBA mice showed that Wv'w subjects have a single haemoglobin band, while CBA and A strain mice have a diffuse haemoglobin pattern comprising two well-separated bands. The faster and major component corresponds in position to the single band of the W series.
TABLE
HAEMATOCRIT VALUES, RED BLOOD COUNTS, AND MEAN CELL VOLUMES OF ADULT A, CBA, AND W^v w STRAIN MICE, AND OF W^v w MICE SUCCESSFULLY TRANSPLANTED WITH A OR CBA HAEMATOPOIETIC TISSUE

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of Animals</th>
<th>Haematocrit Value</th>
<th>Red Blood Count (mm^3 + 10^6)</th>
<th>Mean Cell Volume μ^3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>A strain</td>
<td></td>
<td>11</td>
<td>45.2</td>
<td>40.4-50.4</td>
</tr>
<tr>
<td>W^v w treated with A cells</td>
<td></td>
<td>6</td>
<td>43.6</td>
<td>36.4-48.9</td>
</tr>
<tr>
<td>W^v w untreated</td>
<td></td>
<td>10</td>
<td>38.17</td>
<td>34.7-41.5</td>
</tr>
<tr>
<td>W^v w treated with CBA cells</td>
<td></td>
<td>6</td>
<td>46.5</td>
<td>43.3-48.5</td>
</tr>
<tr>
<td>CBA strain</td>
<td></td>
<td>7</td>
<td>43.9</td>
<td>39.8-49.1</td>
</tr>
</tbody>
</table>

Electrophoresis of the haemoglobins of both the treated series revealed that without exception, in the successfully transplanted animals, the characteristic CBA/A diffuse pattern was present (Fig.).

Discussion
It appears that the heterozygotes (W^v w) of the W-series mice may be transplanted with erythropoietic tissue as readily as the more severely anaemic homozygotes (W^v W^v). The presence of donor type haemoglobin in red cells of all the successfully treated mice long after treatment suggests implantation and continued function of the homologous cells. However, other explanations, such as the transfer of nucleic acids by the donor cells and their incorporation in the host cells, have not been totally excluded.

Summary
Foetal liver cells from either of two haematologically normal strains of mice (A and CBA) were injected at birth into slightly anaemic animals of the genotype W^v w. When adult, these animals had a peripheral blood picture which was typical of the normal donor strain. The electrophoretic pattern of the haemoglobins of both the treated series of W^v w animals was of the diffuse, 2-banded type, of the CBA and A donor strains, instead of the usual single pattern of the W-series. It is implied...
that donor haematopoietic tissue has implanted and is functioning normally in the anaemic animals.

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REFERENCES