Experimental Red Cell Chimerism in the Heterozygote ($W^v w$) of the W-Series Mutants in the House Mouse

MARY J. SELLER

From the Paediatric Research Unit, Guy's Hospital Medical School, Guy's Hospital, London S.E.1

Mice of the genotype $W^v W^v$ 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 $W^v W^v$ 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 $W^v w$, are also anaemic, but are less severely affected than the $W^v W^v$ 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 $W^v w$ animals were treated in a similar manner to the homozygotes $W^v W^v$, 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 $W^v w$ genotype were injected intravenously, within a few hours of birth, with a liver cell suspension (approximately $6 - 10 \times 10^6$ 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% trichloroacetic 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 $W^v w$ animals treated with A foetal liver cells, and the $W^v w$ —CBA treated animals, and a control set of $W^v w$ individuals which had received no treatment, but were matched for age with the test animals.

Results

$W^v w$ animals, aged approximately 18 months, of the colony held by this laboratory were found to have a mean red blood count of 8.84×10^6 cells/mm.³ compared with the normal (approximately 11.5×10^6 /mm.³), and a mean haematocrit value of 38.2%. There was evidence of a slight macrocytosis, the mean corpuscular volume being $43.3 \mu^3$.

Six of the 10 $W^v w$ animals injected with A foetal liver cells, and six of seven of the CBA-treated $W^v w$ 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 haemoglobins of $W^v w$, A, and CBA mice showed that $W^v w$ 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.

Received July 1, 1966.

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

Animals	No. of Animals	Haematocrit Value		Red Blood Count (mm. ³ + 10 ⁶)		Mean Cell Volume μ^3	
		Mean	Range	Mean	Range	Mean	Range
A strain	11	45.2	40.4-50.4	11.14	9.49-12.73	40.3	33.2-47.6
$W^v w$ treated with A cells	6	43.6	36.4-48.9	11.84	10.90-13.65	36.9	31.0-41.7
$W^v w$ untreated	10	38.17	34.7-41.5	8.84	8.09-9.55	43.34	37.6-48.3
$W^v w$ treated with CBA cells	6	46.5	43.3-48.5	11.78	10.55-12.94	39.5	36.1-44.8
CBA strain	7	43.9	39.8-49.1	11.97	11.61-12.61	36.6	32.8-38.9

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

FIG. Electrophoresis on cellulose acetate paper of the haemoglobins of $W^v w$, A, and CBA mice and of $W^v w$ animals successfully transplanted with A or CBA haematopoietic tissue. (Ponceau S.)

that donor haematopoietic tissue has implanted and is functioning normally in the anaemic animals.

This work was supported by the Spastics Society.

REFERENCES

- Bernstein, S. E., and Russell, E. S. (1959). Implantation of normal blood-forming tissue in genetically anemic mice without X-irradiation of host. *Proc. Soc. exp. Biol. (N.Y.)*, **101**, 769.
- Grüneberg, H. (1942). Inherited macrocytic anaemias in the house mouse. II. Dominance relationships. *J. Genet.*, **43**, 285.
- Russell, E. S., and Fondal, E. L. (1951). Quantitative analysis of the normal and four alternative degrees of an inherited macrocytic anaemia in the house mouse. I. Number and size of erythrocytes. *Blood*, **6**, 892.
- Seller, M. J. (1966). Donor haemoglobin in anaemic mice of the W-series transplanted with haematopoietic tissue from an unrelated donor. *Nature (Lond.)*, **212**, 81.
- , and Polani, P. E. (1966). Experimental chimerism in a genetic defect in the house mouse *Mus musculus*. *ibid.*, **212**, 80.