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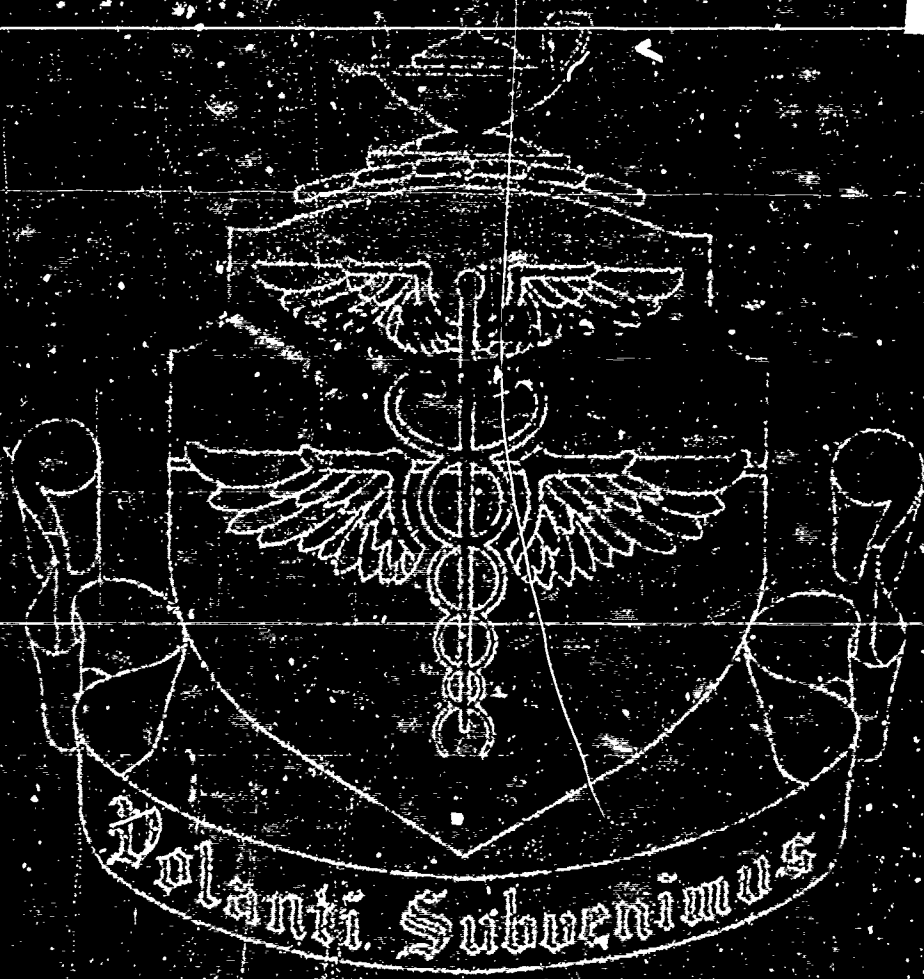
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GENETIC DIFFERENCES IN HEMOGLOBIN AS MARKERS
FOR BONE MARROW TRANSPLANTATION IN MICE

SCHOOL OF AVIATION MEDICINE
RANDOLPH AIR FORCE BASE, TEXAS

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**GENETIC DIFFERENCES IN HEMOGLOBIN AS MARKERS FOR BONE
MARROW TRANSPLANTATION IN MICE**

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GENETIC DIFFERENCES IN HEMOGLOBIN AS MARKERS FOR BONE MARROW TRANSPLANTATION IN MICE

Immunologic, histochemical, and cytologic methods have been used to demonstrate the transplantation and repopulation of erythrocytic (1-4), platelet (5), thymocytic (6), and white blood cell (7, 8) precursors from bone marrow cells of homologous or heterologous origin. The immunohematologic systems now used to type mouse erythrocytes are based on differences in the *H-2* locus (9, 10). This system permits distinction of cells of donor origin from those of the recipient when donor and recipient are of distinguishable *H-2* genotypes. Although chromosomal markers (7) may be used, in cases in which donor and recipient are indistinguishable at the *H-2* locus, the technic cannot be applied to the circulating red cells. An independent method for distinguishing erythrocytes is, therefore, potentially useful in the study of bone marrow transplantation, particularly where the *H-2* markers cannot be used.

With paper electrophoretic technics, Gluecksohn-Waelsch et al. (11) identified inheritable hemoglobin differences in mice. Starch gel electrophoresis, described by Smithies (12), can also be used for identifying hemoglobin differences in mice (fig. 1). This is a preliminary report that indicates that erythrocytes of mouse chimeras can be typed by using inheritable differences occurring in mouse hemoglobin.

METHODS

Mice used as donors and recipients in this experiment were strains C57BL/S, 101, and (C57BL/S × 101)F₁. Erythrocytes of C57BL/S mice are of *H-2^k* genotype and have the "single" type of hemoglobin, whereas erythrocytes of 101 mice seem to be of *H-2^d* genotype and have the "diffuse" type. Erythrocytes of

(C57BL/S × 101)F₁, also have diffuse hemoglobin. Donor bone marrow or splenic suspensions were injected intravenously and intraperitoneally, respectively, into x-irradiated recipients. Blood samples for serologic typing and starch gel electrophoresis were obtained by severing the tail vein.

RESULTS AND DISCUSSION

Table I gives the comparative results obtained in the 20 mice cross-examined by serologic technics (3) and by electrophoresis. The data show that, when bone marrow or splenic suspensions from a donor with one type of hemoglobin are transplanted into irradiated mice that differ at the *H-2* locus and have another type of hemoglobin, the hemoglobin in the recipients' circulating erythrocytes, characterized by starch gel electrophoresis, corresponds with the type of the red blood cells as characterized serologically. The electrophoretic method has not yet been refined sufficiently to detect small quantities (20 percent or less) of the single type hemoglobin when 80 percent or more of the red blood cells have the diffuse type. However, both hemoglobin type and red cell serotype seem to be under the autonomous control of the genotype of the nucleated cell from which the erythrocyte was derived. Makinodan and Anderson (13) also found that hemoglobin in mouse-grown rat erythrocytes has physicochemical properties of rat hemoglobin. Origin (host or recipient) of the circulating erythrocytes can therefore be inferred from the hemoglobin type as well as from the serotype. Thus, after transplantation of bone marrow cells between strains of mice with identical *H-2* genotypes, one can infer whether the circulating erythrocytes are of donor or recipient origin if there are differences in their hemoglobin types; for example, C57BL/6

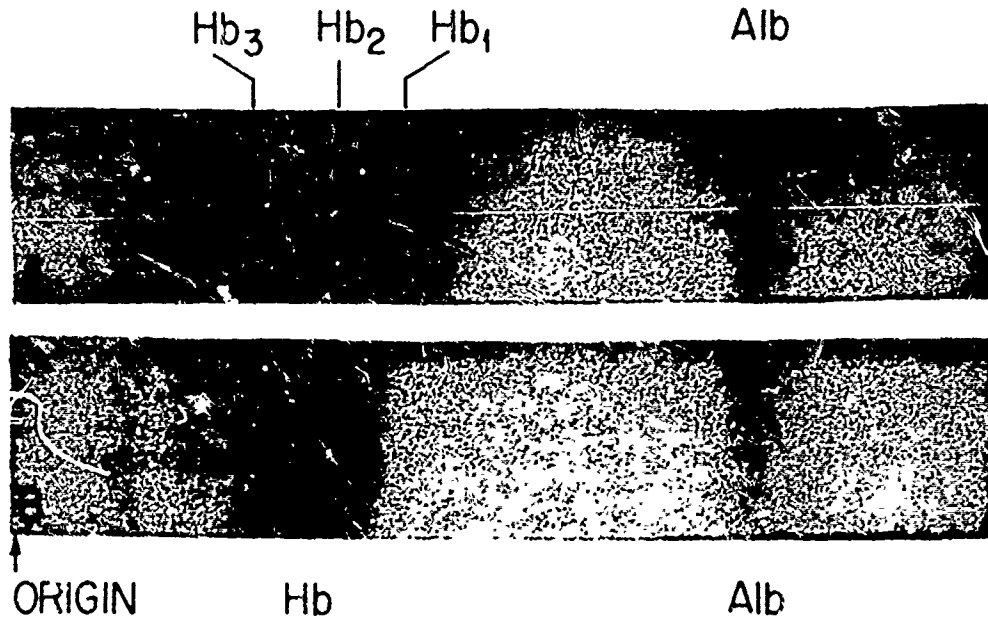


FIGURE 1

Comparative patterns of mouse hemoglobins after electrophoresis in starch gel. Strip A shows the "diffuse" type of hemoglobin of strain 101 mice that resolves into three electrophoretically separable fractions. Strip B illustrates the "single" type of hemoglobin of C57BL/S mice (R. Popp and W. St. Amand, unpublished data).

TABLE I

Comparison of serotype and hemoglobin type in x-irradiate mouse chimeras

| Recipient mice | | Dose (r) | Donor cells ($\times 10^6$ nucleated cells) | | | | Post treatment (days) | Serotype | | Hemoglobin type | |
|----------------|--------------------------------------|----------|--|--------------------------------------|---------|--------------------------------------|-----------------------|---------------|-----------|-----------------|-------|
| | | | Bone marrow | | Spleen | | | Recipient (%) | Donor (%) | Recipient | Donor |
| No. | Strain | | C57BL/S | (C57BL/S \times 101)F ₁ | C57BL/S | (C57BL/S \times 101)F ₁ | | | | | |
| 2 | 101 | 850 | 15 | | | | 43 | 100 | | x | |
| 2 | (C57BL/S \times 101)F ₁ | 900 | 15 | | | | 93 | 100 | | x | |
| 2 | | 900 | 15 | | | | 93 | | 100 | | |
| 1 | | 900 | 15 | | | | 142 | 3 | 97 | | |
| 1 | C57BL/S | 900 | | 15 | | | 42 | | 100 | | |
| 1 | (C57BL/S \times 101)F ₁ | 400 | | | 30 | | 93 | 100 | | x | |
| 1 | | 400 | | | 30 | | 93 | 93 | 7 | x | |
| 1 | | 400 | | | 30 | | 93 | | 100 | | |
| 1 | | 500 | | | 30 | | 21 | 100 | | x | |
| 1 | | 500 | | | 30 | | 21 | 64 | 25 | x | |
| 1 | | 500 | | | 30 | | 21 | 62 | 38 | x | |
| 1 | | 500 | | | 30 | | 21 | 80 | 20 | x | |
| 1 | | 600 | | | 30 | | 93 | 100 | | x | |
| 3 | C57BL/S | 400 | | | | 120 | 35 | 100 | | x | |
| 1 | | 400 | | | | 120 | 35 | 97 | 3 | x | |

and 129/Rr are both *H-2^b*, the former has a single and the latter a diffuse hemoglobin.

Snell et al. (14) and Counce et al. (15) showed that the *H-2* locus strongly affects the transplantability of tumors and skin in mice. Trentin (16) and Uphoff (17, 18) suggested that the *H-2* antigens may also be important in reactions occurring after bone marrow transplantation into lethally irradiated mice. Bone marrow transplantations between sublimes of mice indistinguishable at the *H-2* locus but with different hemoglobin types and varying degrees of heterogeneity at other loci

may further help to elucidate the relative importance of the *H-2* locus and other histocompatibility loci in bone marrow transplantation.

SUMMARY

Both hemoglobin type and red cell serotype seem to be autonomously controlled by the genotype of the nucleated cell from which the erythrocyte is derived. Thus, genetic differences in hemoglobin can be used as markers for bone marrow transplantation in irradiated mice. Hemoglobin typing may be particularly useful where the *H-2* markers cannot be used.

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