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Received February 4, 1964. P.S.E.B.M., 1964, v116.

Correlation Between Genetic Differences and Development of Polycythemia-Anemia in Mouse Parabionts.* (29176)

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F_1 hybrid mice, placed in parabiosis with parental strains, suffer from a disease referred to as parabiotic intoxication(1). These animals have a wasted appearance and are extremely anemic. Billingham(2) has called attention to the similarity between this phenomenon and homologous disease. The latter syndrome can be produced in F_1 hybrid mice inoculated with lymphoid tissue from a parent that differs from the other parental strain at the H-2 histocompatibility locus (3). The injected cells form antibody against the hybrids causing the animals to become anemic and eventually die. Eichwald(1) and his colleagues expressed doubt that this syndrome was comparable to parabiotic intoxication. From their data they concluded that the symptoms of intoxication in parabionts are due to a shift of the red cell mass from intoxicated mice to their pure partners.

Data presented here indicate that a shift of red cells produces anemia and various degrees of polycythemia in both related and unrelated mouse parabionts. Fatal parabiotic intoxication was most severe in the DBA/2 partners of DBA/2:BALB/c parabionts, 2 strains of mice that are identical at the H-2 histocompatibility locus(4).

Materials and methods. Adult mice of both sexes, 3 to 6 months of age, were placed in parabiotic union of the coelomic type as described by Martinez(5). Eight combinations of parabionts were used: 22 pairs of BALB/c:BALB/c; 16 BALB/c:DBA/2; 17 BALB/c:A; 22 BALB/c:C3H; 17 BALB/c:CBA; 18 BALB/c:C57BL/6; 20 BALB/c:C57BR; and 14 BALB/c (tolerant to DBA/2 skin grafts):BALB/c. Parabionts were matched as to sex and weight, housed in 5 × 7-inch plastic cages, and given free access to Purina Laboratory Chow and water. All mice were bled from the retro-orbital sinus at 2-day intervals for a total period of 10 days. Groups of individual animals from each of the strains served as controls and were bled at the same intervals. Blood (0.03 ml) was collected at each bleeding in micro-capillary tubes which were spun in an International Micro-Capillary Centrifuge, model MB, for 5 minutes and read on a Micro-Capillary Reader.

Individual hematocrit readings of the parabionts were combined according to strain and day of bleeding and average values were determined; hematocrits of control groups were averaged in the same manner. Mean hematocrits and standard errors were calculated for day 0, day 4, and day 8.

Results. The hematocrits of the strains of mice differed noticeably (Table I). Russell (6) has reported lower levels for these strains, but the differences may be attributed to alti-

TABLE I. Effect of Continued Bleeding (at 2-day intervals) on Hematocrits of Inbred Strains of Mice.

		Mean hematocrits with standard errors of mean					
		Day 0		Day 4		Day 8	
Strain	No. of Mice	Mean	S.E.	Mean	S.E.	Mean	S.E.
BALB/c	16	50.0	.59	50.2	.54	47.6	.24
A	17	50.8	.78	46.7	1.01	46.2	.81
C3H	15	46.2	.70	44.5	.50	44.4	.95
CBA	20	47.0	.92	44.2	.46	43.0	.49
DBA/2	16	50.5	.47	47.8	.22	43.8	.30
C57BL/6	16	46.9	.39	43.5	.65	43.1	.78
C57BR	16	54.0	.40	50.4	1.42	50.7	1.12

* Supported in part by a research grant from Nat. Cancer Inst., U.S.P.H.S.

TABLE II. Effect of Genetic Differences on Hematocrits of Mouse Parabionts. Each group is in parabiosis with the BALB/c Strain.

Mouse strain	No. of pairs	4th day of parabiosis				8th day of parabiosis			
		BALB/c host		Donor		BALB/c host		Donor	
		Mean hematocrit as % of normal	90% confidence limits for mean hematocrit	Mean hematocrit as % of normal	90% confidence limits for mean hematocrit	Mean hematocrit as % of normal	90% confidence limits for mean hematocrit	Mean hematocrit as % of normal	90% confidence limits for mean hematocrit
BALB/c	22	96.0	89.5-102.5	102.8	96.8-108.8	101.0	97.3-104.7	100.4	94.1-106.7
A	17	96.5	87.0-106.0	92.9	83.3-102.5	99.1	89.3-108.9	77.4	62.9- 91.9
C3H	22	98.6	91.3-105.9	80.8	70.8- 90.8	104.4	93.9-114.9	80.4	66.3- 94.5
CBA	17	101.8	93.7-109.9	80.7	70.6- 92.8	95.1	86.0-104.2	73.0	62.7- 83.3
DBA/2	16	107.4	97.8-117.0	73.8	60.1- 87.5	103.1	90.5-115.7	105.4	93.4-117.4
C57BL/6	18	112.5	108.7-116.3	60.0	51.6- 68.4	123.1	118.3-127.9	74.0	62.5- 85.5
C57BR	20	114.9	109.0-120.8	61.5	54.5- 68.5	131.7	126.7-136.7	62.3	55.4- 69.2

tude. Her study was conducted at sea level while the present study was done at 5000 feet. As expected, a diminution in hematocrits occurred as the result of successive bleedings.

To facilitate comparisons, the means in Table I were treated as "normals" with the appropriate mean hematocrit for the strain and day being taken as 100% of normal for the corresponding parabionts. Using these figures, the hematocrits, as percents of normal, of the parabionts were calculated for the 4th and 8th days; these results are shown in Table II. BALB/c are referred to as "hosts" while their homologous (or isologous) partners are designated "donors."

The BALB/c "hosts" showed a tendency to develop polycythemia that paralleled increasing genetic differences, and the "donor" parabionts became anemic in order of increasing genetic differences from the BALB/c hosts. These changes are clearly seen at the 4th day of parabiosis, a time at which immune reactions would be expected to be at a minimum. At the 8th day the same general pattern remained with the exception of the DBA/2 parabionts; the hematocrits of these animals had returned almost to initial levels or were slightly polycythemic.

In 50% of the BALB/c:BALB/c parabionts, at the 4th day of parabiosis, there was a slight increase in the hematocrits of one of the partners with a decrease in the red blood cell mass of the isologous animals. As indicated (Table II) the 8th day bleedings were approximately equal. There was only a slight change in the A hematocrits of BALB/c:A parabionts on the 4th day but by day 8 the A partners had become anemic. In contrast, DBA/2 partners of BALB/c:DBA/2 parabionts were severely anemic on day 4, but by day 8 had attained almost normal levels or were slightly polycythemic. The closely related strains C3H and CBA showed both an initial and continued shift of the red cell mass to the BALB/c hosts; in addition, the CBA animals showed severe intoxication. Of the 7 strains studied, the DBA/2 were most difficult to keep in parabiosis with BALB/c. Seventy-five percent of the DBA/2 suffered from severe intoxication and died be-

tween the 8th and 10th day of parabiosis. Both C57BL/6 and C57BR strains in parabiosis with BALB/c developed severe anemia on the 4th day of parabiosis; these low hematocrit readings continued throughout the period of observation.

Table I shows the distribution of total hematocrit readings collected from the 7 combinations of mouse parabionts during the 10-day period of observation.

Discussion. On the 4th day of surgical union in both isologous and homologous strains of mice in parabiosis with the BALB/c strain there was a sudden shift of part of the red cell mass from one parabiont to the other. As a result of this action anemia occurred in one animal and polycythemia in the other. In isologous BALB/c parabionts these hematocrit changes returned to normal values by the 6th day of parabiosis. In contrast, the initial as well as a continued shift of red blood cells to the BALB/c animals was apparent in all BALB/c:homologous mouse parabionts. The resultant anemia in the homologous strains, as well as the degree of polycythemia in the BALB/c partners, appeared to increase according to increasing ancestral or genetic differences (Table II).

Four of the strains studied have certain genetic characteristics in common with the BALB/c strain(7). The agouti mice, C3H and CBA, and the albino A have an ancestral line (Bagg albino) in common with BALB/c. The BALB/c mice do not share this common ancestor with the DBA/2 strain and actually differ at many minor histocompatibility loci, but they do have the strong H-2 locus in common. Susceptibility to transplantation appears to be due to multiple dominant histocompatibility genes located at the H-2 locus of chromosomes(8). This locus also determines a red cell antigen and incompatible parabionts could be expected to stimulate the formation of antibodies against red cells. Strains A, C3H, and CBA differ from the BALB/c at this major histocompatibility locus.

The two non-related strains, C57BL/6 and C57BR, have neither breeding ancestry nor histocompatibility alleles in common with the BALB/c which was derived from inbreeding Bagg albinos.

TABLE III. Distribution of Hematocrit Readings Collected from Parabionts from 0 to 10 Days (at 2-day intervals).

Parabionts	No. of pairs	% hematocrits below 45	% hematocrits above 45
BALB/c	22	12.3	87.7
BALB/c		10.0	90.0
BALB/c tol	14	16.0	84.0
BALB/c		24.7	75.3
DBA/2	16	44.7	55.3
BALB/c		18.4	81.6
A	17	45.8	54.2
BALB/c		19.5	80.5
C3H	22	51.0	49.0
BALB/c		23.5	76.5
CBA	17	54.3	45.7
BALB/c		13.3	86.7
C57BR	20	61.1	38.9
BALB/c		1.8	98.2
C57BL/6	18	67.4	32.6
BALB/c		3.0	97.0

The tendency of the red cell mass to shift between the parabionts (Table III) in related strains attests to the compatibility of the pairs. In strains that are not related, C57BL/6 and C57BR, there was a shift in only one direction and the polycythemia that resulted in the BALB/c partners was maintained. It seems apparent that early immune responses in these parabionts sealed off all means of communication between the pairs. In contrast, capillary connections remained open in the related compatible pairs and blood flow between the animals continued for a longer time.

Hematocrit fluctuations (increased hematocrit in one parabiont with a decreased hematocrit in its partner, followed by a reversal at the next bleeding) in related strains might also have been due to intermittent release of red blood cells trapped in the spleen. Spleen weights of parabionts (particularly those of the BALB/c partners in homologous pairs) often averaged as much as 4 times the weight of normal control mice. Microscopic sections showed that the spleens and livers of these mice were engorged with blood(9).

It is difficult to explain the polycythemia that occurred in BALB/c mice in parabiosis with homologous strains. A certain percentage of the homologous parabionts did become

polycythemic, but this number decreased as genetic differences increased between the BALB/c animals and their homologous partners. It would appear that BALB/c mice in parabiosis with homologous animals function as pure mice in parabiosis with F_1 hybrids. Applying Eichwald's hypothesis(1), one could say that the vascular bed of the BALB/c animals dilated and caused an inflow of blood while the capillary vessels of the homologous partners constricted in an attempt to compensate for the loss of red cells. This action resulted in a shift of tissue fluid into the circulation of the homologous animals. The thin, watery appearance of blood samples of mice in parabiosis with BALB/c clearly indicate that this process often occurred.

When DBA/2 mice were placed in parabiosis with BALB/c, the red cell shift to the BALB/c partners occurred in the same manner as in other combinations studied. At approximately 8-10 days there was a change in this usual pattern and a majority of the DBA/2 animals either showed approximately the same hematocrit as the BALB/c partners (normal hematocrits) or were slightly polycythemic. These sudden hematocrit changes could have contributed to the cause of the high death rate of the DBA/2 animals in this combination of parabionts. Another cause of death might be found, ironically, in actual genetic similarity. Since both animals belong to the same H-2 (H-2d) histocompatibility locus, capillary anastomoses between these strains would remain open and permit antigen-antibody reactions to act for a longer period than between strains which did not belong to the same H-2 locus as the BALB/c. That the DBA/2 animals were the first to die suggests that the BALB/c strain possesses much stronger inherent physiological and immunological capabilities.

In the BALB/c tolerant:BALB/c parabionts the role of the pure partners appeared to be reversed with the red cell shift favoring the tolerant mice at the 4th day of parabiosis. Presumably the DBA/2 and BALB/c white cells of the chimaeras were tolerant to each other, and, as a consequence of such neutralization, could not precipitate the physiological shock that the BALB/c animals experience on parabiosis with immunologically competent homologous strains.

The data presented here indicate that genetic differences between BALB/c mice in parabiosis with homologous strains contribute to the stimulus that precipitates the shift of red cells between parabionts. Strong genetic differences encourage a massive shift of the red cell population to the BALB/c partners.

Summary. 1. BALB/c mice were placed in parabiotic union with 4 related (A, C3H, CBA, and DBA/2) and 2 unrelated (C57 BL/6 and C57BR) strains of mice. Hematocrits were read at 2-day intervals for a total period of 10 days. 2. The BALB/c parabionts showed the same or an increased hematocrit at the 4th day of parabiosis while their homologous partners showed varying degrees of anemia. 3. Hematocrit differences between the BALB/c and homologous parabionts increased with increasing genetic differences. 4. Seventy-one percent BALB/c mice (tolerant to BALB/c skin grafts) in parabiosis with isologous mice showed increased hematocrits at 4 days. This increase was maintained in 35% of the tolerant pairs.

The author wishes to express appreciation for technical assistance to Andrea Bretag, Chester Cook, and Sharon Atkinson.

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Received February 4, 1964. P.S.E.B.M., 1964, v116.