

Department of Surgery, The Institute of Medical Science,
The University of Tokyo, Tokyo Japan.

IMMUNOPATHOLOGICAL STUDIES OF PARABIOSIS IN ADULT MICE

By

KAZUTAKA ASHIKAWA, KIICHI INOUE &
YASUZO H YOSHIDA

INTRODUCTION

The failure to obtain successful homografts among non-related individuals of the same species is due to antigenic differences occurring between the donor and the recipient on the basis of which the specific antibody reaction results.

It has been recognized that the best method opposing to tissue incompatibility is the experimentally acquired tolerance of foreign antigens elicited by an organism in its early antigenic stages. This can be accomplished by injection of homologous cells to the fetus or new-born animals or by the method of experimental parabiosis shortly after the birth. Erythrocyte chimera between dizygotic cattle twin described by *Owen* (25), is the result of natural embryonic parabiosis in which the embryonic blood is exchanged between the partners due to vascular anastomosis. It can be said that the method used for the induction of acquired immunological tolerance by *Billingham* (5) is always more or less an imitation or artificial reproduction of such a spontaneous parabiosis.

Martinez (19) showed that tolerance induced in adult mice by establishing a parabiotic union between the members of two homologous strains could be transferred to isologous animals by injection of spleen cells to these mice from tolerant mice at birth. Such a phenomenon suggests that parabiotic union induces cellular chimera in spite of an existence of genetic difference between donors and hosts. Those

animals which regressed transplanted tumor cells usually have an immunological resistance to reinoculation of the same tumor cells. The role of humoral or cellular antibodies in such an immunity is now discussed among various investigators.

Studies of parabiosis have been thought to be useful to solve this problem. However, various investigators have reported different results and opinions. Transfer of immunity to normally susceptible animals by parabiosis has been reported by some authors (3, 4, 12, 20), whereas other reports seem to demonstrate the contrary (1, 8, 14). One author described that immunity was actually converted to susceptibility (9). Such seemingly conflicting results might come from the genetic type of animals used. By means of labelling red blood corpuscles it was observed that crossed blood flow in homologous pairs was approximately one hundred times that in pairs which were heterogenic. *Nakic* (22) showed that whole antigen in both homologous parabiotic partners is continuously replaced in a form of „cells“ and then cellular chimera is established.

However, so-called „competitive replacement hypothesis“ postulated by *Nakic* still remains a matter of debate. Our experiments presented here shows that tolerance can not always be produced in all parabiotic combinations, even when the H-2 types are the same. In the parabiosis between parents and F₁ hybrid animals, recolonization of F₁ cells to parental partner is interrupted by the immune response of the host against F₁-antigens which appear to prevent the induction of a state of tolerance.

MATERIAL AND METHODS

1. *Animals*: 4-8 week old C₃H/He, DDD, C₅₇BL, NC, FM, AKR and (DDD ♀ × C₃H/He ♂) F₁ hybrid mice weighing 18-23 gm were obtained from the experimental animal room in our institute.

2. *Parabiosis*: Parabiotic union was carried out by the original technique of *Bunster & Meyer* (7). Parabiont mice were anesthetized with an intraperitoneal injection of pentobarbital and shaved on the appropriate lateral surface of the trunk.

A linear incision extending from the base of ear to the base of thigh was made in each of the two animals. Skin flaps were raised for a width of 1-2 cm and the flaps of the scapulae adjacent to the incision were dissected free of surrounding musculature. The peritoneum was incised from the femoral region up to the level of the eleventh rib; care was taken to avoid entering the pleural cavity. All four peritoneal edges were joined with a continuous silk suture to obliterate communication between the coelomic cavities. This prevented obstruction secondary to migration and entanglement of intestinal loops yet provided an additional layer of united tissue in which vascular communications could develop. The scapulae were joined with silk to prevent cutaneous tension and wound breakdown when the mice tried to turn in the opposite directions. The skin flaps then were joined both ventrally and dorsally with silk suture. We also used the technique of *Sauerbruch & Heyde* (27) in which the peritoneal cavities of the two parabiont mice were not opened and only skin flaps were sutured together.

3. *Red and white blood cell count:* The examined blood samples were obtained from the right axillary artery of the parent-F₁ parabionts to evaluate blood cell count.

4. *Histopathological examination:* Histopathological examination was performed on the spleen, the lymphatic tissue, the liver and the kidneys of each animal following parabiosis. The specimen was stained with hematoxylin-eosin.

5. *Paper electrophoresis:* Paper electrophoretic analysis was carried out by the method of *Mori et al.* (21). Filter paper strips (12.0 × 2.5 cm) should be wet with veronal buffer solution at pH 8.6 (ionic strength 0.02) and placed in the apparatus on a siliconized polished glass. The sample was applied as a narrow band with a capillary pipet. Limiting current flow to 2 ma. per strip at 400 volt was used for 6 hours. The dried paper strips were stained with bromphenol blue and the electrophoretic pattern was calculated by using a densitometer. Protein components obtained in this way were shown as percentage.

6. *⁶⁰Co irradiation:* Mice were put in a round plastic cage and 500-800 r of ⁶⁰Co was irradiated to the whole bodies of mice.

7. *Skin homografts:* Skin homografting was performed by the technique of *Billingham & Medawar* (6), basically. The operated areas of both donor and recipient mice were depilated with depilatory cream 2-3 days prior to skin grafting. Full-thickness and round grafts, 12 mm in diameter were removed from the donor mice anesthetized with subcutaneous injection of 0.03-0.04 ml of Nembutal® (Pentobarbital sodium—50 mg/dl) obtained from Abbott Product, U.S.A. and placed on a prepared graft bed in the back of the recipient. These grafts were fixed with Aron-alpha. A obtained from the Toa Gosei Chemical Industry, Tokyo, Japan and Sofra-Tulle gauze obtained from Roussel Laboratories, London, England and sterilized gauze (3.0 × 3.0 cm) were applied with vinyl adhesive tape. When approximately 50 per cent of the area of a graft was occupied with such signs as cyanosis, edema, petechiae, induration and

necrosis of grafts, the graft was judged as „rejected“. From one week after skin grafting, the graft survival was observed every day.

8. *Bone marrow cells*: In order to obtain bone marrow cells of mouse, each edge of the humerus and the femurs was cut, then 1/1 i.v. needle was inserted into the bone marrow cavity. After several times of „pumping“ through the bone marrow cavity, the dissociated cell suspension was intravenously injected from the tail vein of mice as slowly and gently as possible.

9. *Ultra-sonic preparation of the bone marrow cell suspension*: Bone marrow cell suspension obtained as mentioned above was treated for 10 minutes at 10 Kv, 1.5 ma. No formed cells were microscopically observed in the treated bone marrow cell suspension.

10. *Normal mouse serum*: Blood was taken from the right axillary artery of C₃H/He mice and the pooled blood was centrifuged at 3,000 r.p.m. for 30 minutes. 0.5 ml of the serum was intravenously injected from the tail vein of recipient mice.

11. *Tumor cells*: Spontaneous mammary carcinoma, MH134 originated from C₃H/He mice was changed into ascitic type and maintained at the Department of Carcinostatics in our institute. 2.0×10^6 of MH134 cells were subcutaneously inoculated to recipient mice.

12. *Measurement of complement*: Complement level was measured by modified Mayer's method. The same amounts of individually separated sera of one group containing 5 mice were pooled together for measurement of complement level. Sensitized erythrocytes and complement were mixed at an interval of 3 minutes. 5×10^7 of properly sensitized erythrocytes were shaken in 7.5 ml of veronal buffer solution including Ca^{++} and Mg^{++} for 90 minutes at 37° C. When 50 per cent (2.5×10^7) of these sensitized erythrocytes were hemolysed, its complement level was regarded as C'H₅₀ units.

13. *Hemolysin test*: Dilutions were made of mouse anti-sheep erythrocyte stromata and 0.25 ml portions were added to 0.25 ml of sheep erythrocyte suspension, 1×10^8 per ml. After shaking for 20 minutes at 37° C, 0.5 ml of 1/25 dilution of guinea-pig complement previously absorbed nine times with packed sheep erythrocytes at 0° C to remove „natural“ hemolysins were added to each mixture. After shaking for 90 minutes at 37° C, each mixture was centrifuged at 2,000 r.p.m. for 20 minutes to settle down residual erythrocytes. The degree of lysis were visually estimated. Scoring was based on a system of „0“ (no hemolysis) to „4“ (complete hemolysis).

RESULTS

1. *Duration of Parabiotic Union in Normal Adult Mice*:

Spontaneous separation of union or so-called „parabiotic disease“ occurred 7–15 days (average: 12.3 days) following

TABLE 1
Parabiosis in the adult mice.

Combination		H-2 barrier	Duration of parabiosis time (days)
C ₃ H/He	: DDD	+	7-15
C ₃ H/He	: C ₅₇ BL	+	7-16
NC	: C ₅₇ BL	+	6-60
FM	: C ₃ H/He	+	7-14
DDD	: DDD	—	>180
C ₃ H/He	: C ₃ H/He	—	>180
AKR	: C ₃ H/He	—	12-96

parabiosis between allogenic combination such as C₃H/He-to-DDD.

The C₃H/He-to-C₅₇BL parabiotic partners showed such a phenomenon on 7-16 days (average: 13.1 days), NC-to-C₅₇BL, 6-60 days (average: 26.4 days) and FM-to-C₃H/He, 7-14 days (average: 11.4 days), respectively. The prolongation of parabiotic union was seen in the groups of mice of which H-2 loci were the same. The AKR-to-C₃H/He (H-2K) parabiotic mice showed the duration of union of 12-96 days and AKR-to-AKR or C₃H/He-to-C₃H/He partners mostly (80 per cent) maintained the unions over a period of 180 days.

2. Duration of Parabiotic Union in F₁ Hybrid to Parent Mice:

The difference of parabiotic duration was not significant in parabiosis between (DDD ♀ × C₃H/He ♂) F₁ hybrid and its parental C₃H/He.

On the contrary, when this F₁ hybrid was joined with another parental strain, DDD, the duration of parabiotic union showed a significant prolongation ranging from 10 to 100 days. In the most F₁ hybrid mice clinical signs of parabiotic disease such as failure to thrive, diarrhea, alopecia, etc were observed, and approximately 60 per cent of them died at the

TABLE 2
Electrophoresis pattern in F₁ and C₃H.

Mice	Protein	Range	Mean
(C ₃ H/ × DDD) F ₁	Albumin	57.5-66.3 %	62.5 %
	Alpha-Globulin	12.0-15.2	13.5
	Beta-Globulin	13.6-21.8	16.7
	Gamma-Globulin	4.0-12.0	7.2
	Total Protein	6.4- 7.2	6.9 g/dl
C ₃ H/He	Albumin	37.4-45.2	42.2
	Alpha-Globulin	19.5-24.6	22.7
	Beta-Globulin	25.0-33.4	27.4
	Gamma-Globulin	6.2-10.3	8.0
	Total Protein	6.0- 8.0	6.8 g/dl

end of 2 weeks or early in 3 weeks after joining with its parental mice.

3. *Changes of Peripheral Blood Count Following F₁-to-Parent Parabiosis:*

Patho-physiological observations have been performed in the F₁-to-its parental C₃H/He parabiotic mice. A fall of white blood cells in the peripheral blood of F₁ partner (2,000/ μ l) occurred at the second day of parabiosis and this was followed by a rapid rise up to 9,600/ μ l 4 days after parabiosis. Then, they showed a tendency of decrease. Red blood cells in the peripheral blood of F₁ partner were inclined to decrease day by day and the color of the skin became pale.

4. *Changes of Electrophoretic Pattern of Serum Protein Following Parabiosis:*

The paper electrophoretic pattern of serum protein in C₃H/He and F₁ hybrid was as follows: In F₁ hybrid mice albumin was 57.5-66.3 per cent (average: 62.5 per cent); alpha-globulin 12.0-15.2 per cent (average: 13.5 per cent); beta-globulin 13.6-21.8 per cent (average: 16.7 per cent);

TABLE 3

Electrophoresis pattern in parabiosis mice.

Protein	Mice	Days after parabiosis			
		2	4	6	8
Albumin	C ₃ H/He	59.3 %	62.6 %	59.9 %	62.2 %
	F ₁	52.4	59.9	61.6	51.9
Alpha-Globulin	C ₃ H/He	17.4	15.6	11.0	15.1
	F ₁	21.4	17.6	12.7	14.5
Beta-Globulin	C ₃ H/He	21.0	17.4	21.1	18.2
	F ₁	21.4	16.2	18.0	29.0
Gamma-Globulin	C ₃ H/He	2.2	4.2	8.0	4.4
	F ₁	4.9	3.9	7.6	4.5
Total Protein	C ₃ H/He	5.6 g/dl	6.0 g/dl	5.6 g/dl	5.8 g/dl
	F ₁	6.3	6.0	5.7	6.4

gamma-globulin 4.0–12.0 per cent (average: 7.2 per cent) and total protein 6.4–7.2 gm/dl (average: 6.9 gm/dl). C₃H/He mice showed 37.4–45.2 per cent (average: 42.2 per cent) of albumin, 19.5–24.6 per cent (average: 22.7 per cent) of alpha-globulin, 25.0–33.4 per cent (average: 27.4 per cent) of beta-globulin, 6.0–10.3 per cent (average: 8.0 per cent) of gamma-globulin and 6.0–8.0 gm/dl (average: 6.8 gm/dl) of total protein. The electrophoretic pattern of serum protein was examined on the second, fourth and eighth day of parabiosis.

C₃H/He mice showed an increase of albumin on the second day of parabiosis and it approximated to the same value on the eighth day of parabiotic union. On the contrary, F₁ hybrid mice maintained normal pattern of albumin and even on the eighth day of parabiosis, it showed no significant changes. Alpha-globulin of the C₃H/He partner had almost normal values on the second day of parabiosis and thereafter it had a tendency towards depression. However, the F₁ partner showed an increase of alpha-globulin from the beginning. C₃H/He mice tended to decrease 2, 4 and

TABLE 4
Spleen weight of parabiosis mice.

Treatment	Mice	Spleen weight (g)
None	C ₃ H/He	0.124
	DDD	0.143
	F ₁	0.153
⁶⁰ Co 500 r Irrad.	F ₁	0.078
F ₁ (500 r) : DDD	DDD	0.158
Parabiosis	F ₁	0.296
F ₁ (500 r) : C ₃ H/He	C ₃ H/He	0.054
Parabiosis	F ₁	0.144

6 days after parabiosis and then showed an increase above normal limits. Beta-globulin in the F₁ partner constantly increased. In both partners, gamma-globulin had a low level until on day six when it showed a temporary increase. However, it decreased gradually from the eighth day of parabiosis.

5. *Changes of Relative Weight of the Spleen After Parabiosis:*

The average spleen weight of normal adult C₃H/He, DDD and F₁ hybrid mice were 0.124 gm, 0.143 gm and 0.153 gm, respectively.

One week after whole body irradiation of ⁶⁰Co (500 r), F₁ hybrid spleen weighed 0.078 gm which was almost equal to half of the normal spleen weight. F₁ hybrid mice irradiated with a similar dosage of ⁶⁰Co were parabiosed with DDD mice immediately after irradiation. The average spleen weight of such F₁ hybrid mice was 0.158 gm on the seventh day of parabiosis and that of DDD-parabionts weighed about twice the normal level (0.296 gm). When F₁ hybrid mice irradiated in the same way were joined with their parental C₃H/He mice, the spleen weight of the F₁ hybrid mice was 0.054 gm

HYBRID-PARENT PARABIOSIS IN MICE

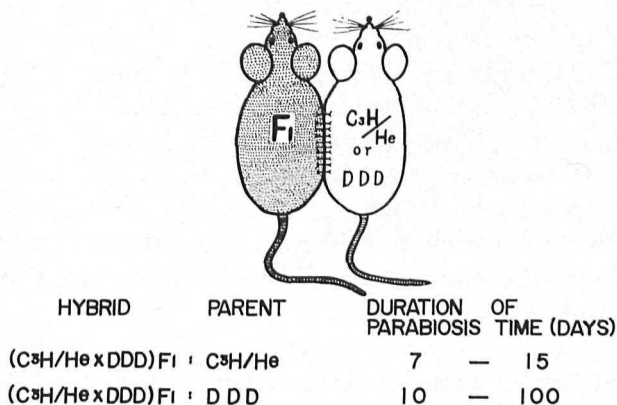


Fig. 1.

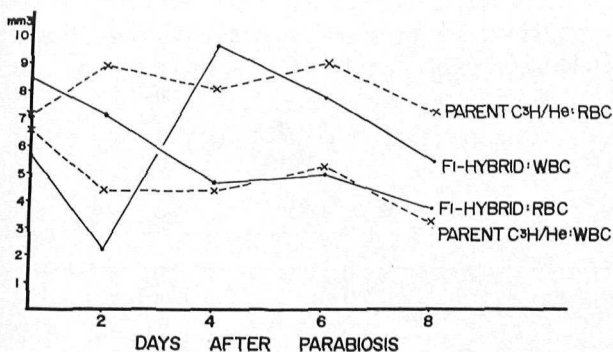
RBC AND WBC COUNTS IN F₁-HYBRID TO PARENT C³H/He AFTER VARYING INTERVALS OF PARABIOSIS

Fig. 2.

(1/3 of normal mice) and that of C³H/He mice weighed 0.144 gm (almost normal level). The spleen weight of F₁ hybrids showed a significant difference by changing parabiotic partners. This difference may be due to development of parabiotic disease in F₁ hybrid-DDD parabiosis more slowly than that in F₁ hybrid-C³H/He parabiosis.

6. *Histological Examination of the Thymus After Parabiosis:*

Fig. 1 and Fig. 2 are microphotographs of histological findings of the thymus in a parabiotic F₁ hybrid and its parental C₃H/He one week following parabiosis. Although significant decrease of parenchymal cells and proliferation of eosinophilic cells were observed in F₁ hybrid mice, C₃H/He mice showed no significant changes. F₁ hybrids also exhibited remarkable atrophy of the lymphatic tissues. However, it was impossible to find significant changes in the liver, the kidney, the adrenal glands, and other organs of both partners.

7. *The Survival Time of Skin Homografts in Adult Mice:*

The skin homografts of normal mice showed survival time as follows. The skin grafts from C₃H/He to DDD survived for 9–13 days (average: 11.6 days) and that from DDD to C₃H/He rejected 9–13 days after skingrafting (average: 11.2 days). When skin-grafting was performed from the parental DDD to F₁ hybrid, the grafts showed the development of donor's white hair after about 20 days and these grafts survived for over one year. A similar phenomenon was observed in the grafts from the parental C₃H/He to F₁

TABLE 5
Survival times of skin homografts.

Donor	Recipient	Graft survival	
		Range	Mean
C ₃ H/He	→ DDD	9–13	11.6
DDD	→ C ₃ H/He	9–13	11.2
DDD	→ F ₁		> 180
C ₃ H/He	→ F ₁		> 180
F ₁	→ DDD	13–64	37.2
F ₁	→ C ₃ H/He	17–78	34.8
F ₁	→ F ₁		> 180
C ₃ H/He	→ C ₃ H/He		> 180
DDD	→ DDD		> 180

hybrid. On the contrary, the skin grafts from F₁ hybrid to the parental DDD mice were rejected 13–64 days (average: 37.2 days) after skin-grafting and those from F₁ hybrid to C₃H/He were rejected 17–78 days (average: 34.8 days) after skin transplantation. The evidence of rejection was not observed for one year and more in the grafts from F₁ hybrid to F₁ hybrid or DDD to DDD.

8. *The Survival Time of the Skin Grafts After Parabiosis:*

The skin-grafting was performed from DDD mice immediately after parabiosing F₁ hybrid with DDD. These grafts survived during the continuance of parabiotic union. When the grafts were transplanted from C₃H/He immediately following F₁ hybrid-DDD parabiosis, F₁ hybrid mice accepted the grafts during the persistence of parabiotic union. However, DDD-partners rejected the grafts 9–27 days (average: 14.8 days) following transplantation. The skin

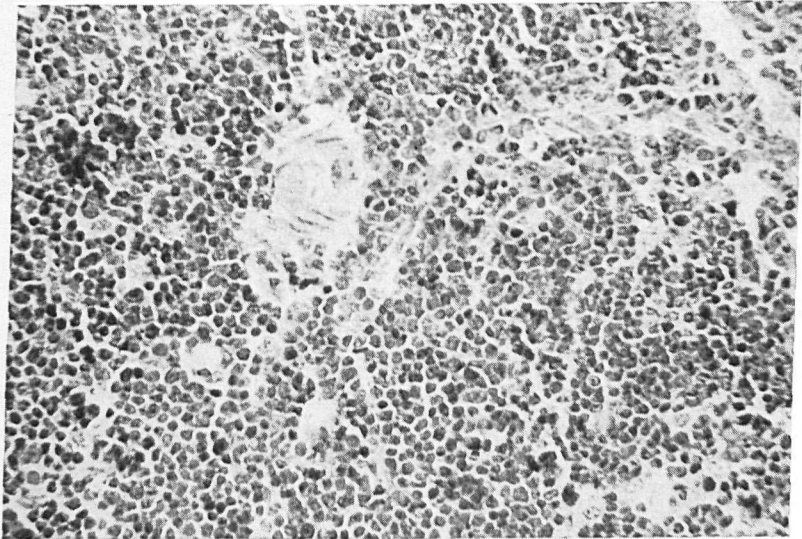


Fig. 3.

Thymus of parental C₃H/He mouse (40 × 10) after parabiosis.

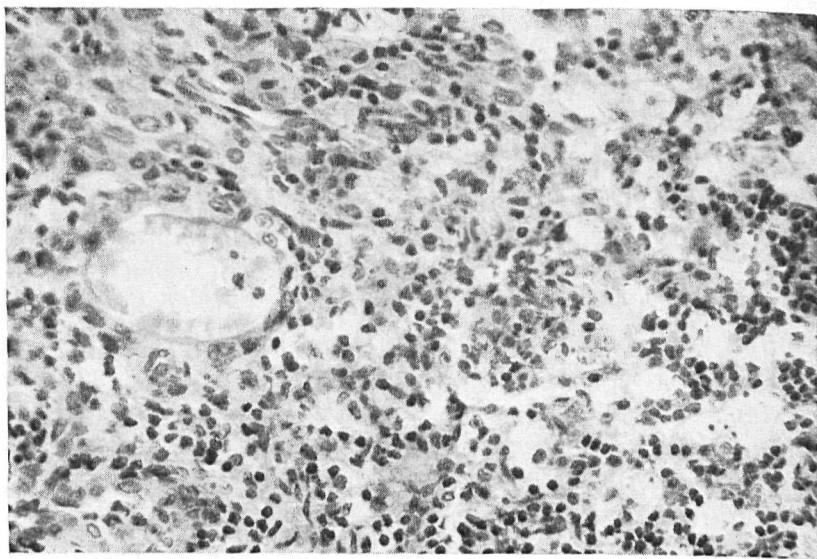


Fig. 4.

Thymus of F₁ hybrid mouse (40 × 10) after parabiosis.

grafts from F₁ hybrids were transplanted to DDD and those from DDD were put on F₁ hybrids following F₁ hybrid-DDD parabiosis. In such groups, F₁ hybrids accepted the grafts from DDD; however, DDD rejected the grafts from the F₁ hybrid partner within 7–16 days after grafting (average: 8.7 days).

9. *The Survival Time of the Skin Grafts after Parabiosis with ⁶⁰Co Irradiation:*

The skin grafts from DDD to C₃H/He showed a mean survival time of 9–13 days (average: 11.2 days) and those from DDD to the recipient C₃H/He irradiated 600 r of ⁶⁰Co survived for 9–26 days (average: 18.5 days). C₃H/He and F₁ hybrid were parabiosed immediately after irradiation (⁶⁰Co 600 r) and the skin grafts from DDD were transplanted. In four pairs of ten pairs, DDD-grafts survived for over 60 days.

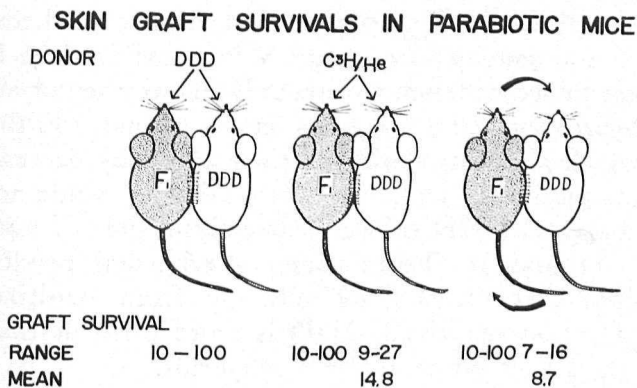


Fig. 5.

10. *Transfer of Anti-Sheep Erythrocyte Antibody by Parabiosis:*

0.5 ml of sheep erythrocytes suspension (10^9 cells equivalent) was injected into the tail vein of DDD mice. One week following this, the same amount of sheep erythrocytes suspension was injected again for sensitization. Four days after the booster injection, parabiosis was performed between these sensitized DDD mice and non-sensitized F₁ hybrid mice. 2, 4, 6 and 9 days after parabiosis, a blood sample was drawn from 3 parabiotic pairs. Serving as controls was a group of sensitized DDD-to-sensitized DDD combination from which blood was taken 2 days after parabiosis. The results are shown in Fig. 4.

Hemolysin titer showed 6,400 units in sensitized DDD-sensitized DDD parabionts and DDD not undergoing parabiosis. However, hemolysin titer revealed 3,200-6,400 units in the sensitized DDD-to-non sensitized F₁ hybrid parabionts 2-11 days after parabiosis. Sheep erythrocyte antibody was passively transferred from sensitized DDD to F₁ hybrid. In the same experimental system, sensitized F₁ hybrid-to-non sensitized DDD parabiosis showed the hemolysin titers presented in Fig. 5.

Sensitized F₁ hybrid without parabiosis and sensitized F₁

hybrid-sensitized F₁ hybrid parabionts showed a hemolysin titer of 6,400 units on an average. When sensitized F₁ hybrid mice were joined with non sensitized DDD, the hemolysin titer of F₁ hybrid showed 1,600 units on day second, fourth, sixth and ninth day of parabiosis and then gradually decreased to 800 units on day 11 and 400 units on day 16, while non sensitized parental DDD showed a hemolysin titer of 240 units on day 11 and 16. From these experimental results it is considered that transfer of antibody from sensitized F₁ hybrids to non sensitized DDD is more difficult than that from non sensitized DDD to F₁ hybrids.

11. *Changes of Complement Level by Parabiosis:*

The complement level of normal adult F₁ hybrid mice was 72.0–77.0 (average: 74.5) and that of DDD mice was below 20.0. Therefore, it should be possible to analyze exchanging mechanisms of humoral factors by showing fluctuations in complement titer following parabiosis. 2, 4, 6, 7, 14, 21 and 28 days after F₁ hybrid-to-DDD parabiosis, the complement titer was measured. As shown in Table 6, F₁ hybrids showed a complement level of 31.0–38.0 (average: 35.0) 2 days following parabiosis, 30.0–38.0 (average: 34.0) 4 days, and 27.0–43.5 (average: 38.0) 6 days after parabiosis. 2, 4 and 6 days after parabiosis, the complement titer of DDD mice was below 20.0. One week after parabiosis, the complement titer of F₁ hybrid mice was 29.0–60.0 (average: 44.5);

TABLE 6
*Tolerance of hybrid-parent parabiosis
combined with ⁶⁰Co total-body irradiation.*

Donor	Recipient	Graft survival	
		Range	Mean
DDD	C ₃ H/He	11.2	9–13
DDD	C ₃ H/He (⁶⁰ Co 600 r)	18.5	9–26
DDD	→ F ₁		>60
DDD	→ C ₃ H/He (⁶⁰ Co 600 r)		>60

TABLE 7
Serum complement level in parabiotic mice.

Days after separation	F ₁ -hybrid	Parent-DDD
2	35.0 (31.0-38.0)	<20.0
4	34.0 (30.0-38.0)	<20.0
6	38.0 (27.0-43.5)	<20.0
1 week	44.5 (29.0-60.0)	54.5 (49.0-60.0)
2 weeks	37.0 (36.0-55.0)	48.5 (48.0-48.5)
3 weeks	54.0 (41.0-54.0)	41.0 (31.0-47.0)
4 weeks	48.0 (41.0-54.0)	42.0 (40.0-44.0)
Normal mice	74.5 (72.0-77.0)	<20.0 (20.0-20.0)

however, that of DDD was 49.0-60.0 (average: 54.5). Two weeks after parabiosis, the complement titer of DDD mice showed a rather higher value (48.0-48.5, average: 48.5) than that of F₁ hybrid mice (36.0-55.0, average: 37.0). Three weeks after parabiotic union, the average complement level was 54.0 in F₁ hybrid mice and 41.0 in DDD mice. Four weeks after parabiosis, F₁ hybrid mice had a complement titer of 48.0 on an average, and DDD mice averaged almost the same titer of 42.0. F₁ hybrid-to-DDD parabionts were separated 2 weeks following parabiosis. Two, 4, 7 and 9 days after separation of parabiotic union, their complement levels were measured. As presented in Table 7, the complement titer of F₁ hybrid mice 2, 4, 7 and 9 days after separation of union was 48.0, 68.0, 65.0 and 53.0, respectively.

TABLE 8

Serum complement level after separation in parabiotic mice.

Days after parabiosis	F ₁ -hybrid	Parent-DDD
2 days	48.0 (44.0-52.0)	< 20.0
4 days	68.0 (67.0-69.0)	< 20.0
7 days	65.0 (63.5-67.0)	< 20.0
9 days	53.0 (48.5-58.0)	< 20.0
Normal mice	74.5 (72.0-77.0)	(20.0-20.0) < 20.0

This complement level of DDD mice rapidly returned to the preoperative value on the second day of parabiotic separation.

12. *Changes of Serum Complement by the Bone Marrow Transplantation:*

A gradual decrease of complement titer in C₃H/He mice following 800 r of ⁶⁰Co irradiation is demonstrated in Fig. 6. 2.0×10^7 of normal isologous bone marrow cells were intravenously injected into the tail vein 3 times at 0, 24 and 48 hours after irradiation.

The complement titer of such treated mice revealed the lowest value on the seventh day after irradiation and then showed gradual recovery. The same amount of bone marrow cells treated with ultrasonics or normal serum of C₃H/He mice with a sufficient complement titer were intravenously injected into the irradiated mice.

There was no significant difference in complement titer between such treated mice and non treated (only irradiated) mice. The animals inoculated with 9.0×10^7 bone marrow

HEMAGGLUTININ ANTIBODY LEVEL IN PARABIOTIC MICE

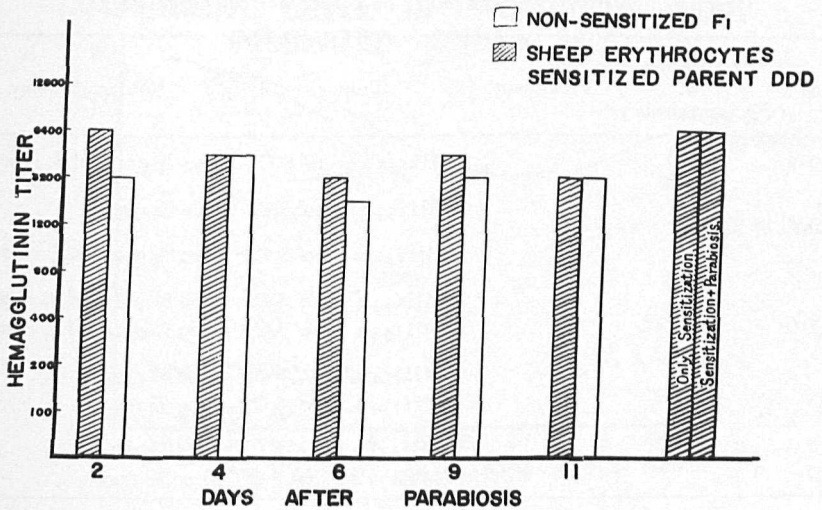


Fig. 6.

HEMAGGLUTININ ANTIBODY LEVEL IN PARABIOTIC MICE

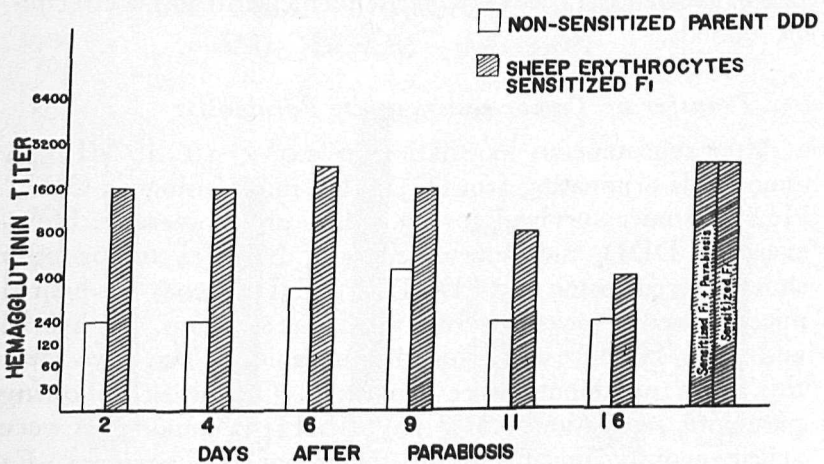


Fig. 7.

TABLE 9

Transfer of anti-tumor resistance in adult mice by parabiosis.

Mice	Duration of parabiosis	Treatment	Tumor	Course of tumor
C ₃ H/He			MH ₁₃₄ 2×10^6 S.C.	100 % prog. M.S. = 23.0
DDD			MH ₁₃₄ 2×10^6 S.C.	100 % reg.
F ₁			MH ₁₃₄ 2×10^6 S.C.	100 % prog. M.S. = 28.3
F ₁	10 days		MH ₁₃₄ 2×10^6 S.C.	100 % prog. M.S. = 31.5
C ₃ H/He			MH ₁₃₄ 2×10^6 S.C.	100 % prog. M.S. = 25.1
F ₁	20 days		MH ₁₃₄ 2×10^6 S.C.	100 % reg.
DDD			MH ₁₃₄ 2×10^6 S.C.	100 % reg.
F ₁	10 days	⁶⁰ Co 500 r	MH ₁₃₄ 2×10^6 S.C.	35 % reg.
DDD			MH ₁₃₄ 2×10^6 S.C.	40 % reg.

cells showed more rapid recovery in complement titer than those inoculated with 6.0×10^7 of bone marrow cells as shown in Fig. 7. Thus, the number of inoculated bone marrow cells paralleled the recovery of complement titer in the recipient animals.

13. *Transfer of Tumor-resistance by Parabiosis:*

After subcutaneous inoculation of 2.0×10^6 of MH₁₃₄ tumor cells originating from C₃H/He mice, isologous C₃H/He strain mice survived for 23.0 days on an average. However, all DDD mice inoculated with MH₁₃₄ tumor cells showed a regression and (DDD ♀ $\times$ C₃H/He ♂) F₁ hybrid mice showed a mean survival time of 28.3 days. F₁ hybrid and C₃H/He mice were joined in parabiosis. Ten days after this, both parabionts were separated. One week following parabiotic separation, 2.0×10^6 of MH₁₃₄ tumor cells were subcutaneously inoculated to the separated partners. F₁ hybrid mice had a mean survival time of 31.5 days and C₃H/He mice of 25.1 days. Twenty days after F₁ hybrid-

EFFECT OF PASSIVE CELL TRANSFER ON COMPLEMENT LEVEL IN ^{60}Co TOTAL BODY IRRADIATED $\text{C}^3\text{H}/\text{He}$ MICE

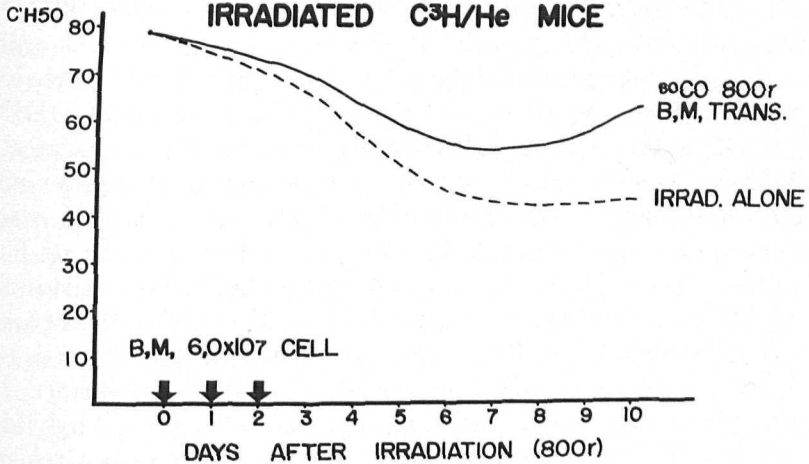


Fig. 8.

COMPLEMENT LEVEL OF ^{60}Co TOTAL-BODY IRRADIATED $\text{C}^3\text{H}/\text{He}$ MICE FOLLOWING BONE MARROW CELL TRANSFER, DESTROYED CELL AND SERUM INFUSION

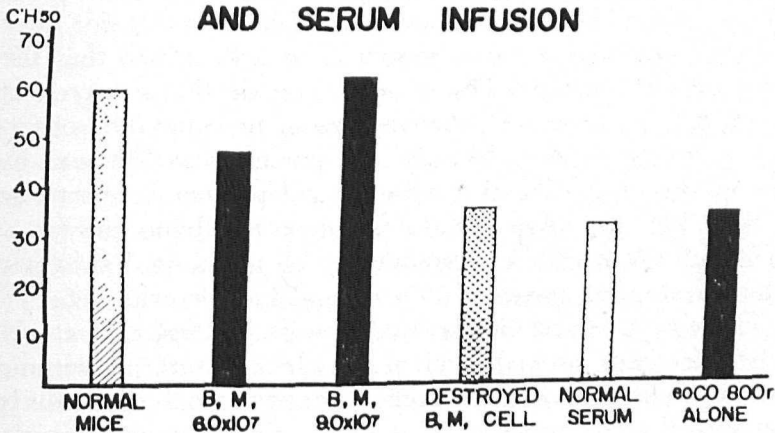


Fig. 9.

to-DDD parabiosis, the parabiotic union was separated. One week following separation, 2.0×10^6 of MH134 tumor cells were subcutaneously injected to both partners. DDD mice rejected MH134 cells at the rate of 100 per cent and F1 hybrid mice showed regression in 35 per cent. F1 hybrid mice irradiated with 500 of ^{60}Co were parabiosed with DDD mice. Ten days after parabiosis the parabionts were separated. MH134 tumor cells were inoculated one week following parabiotic separation. Thereafter, DDD mice showed 100 per cent of regression of MH134 cells and 40 per cent of F1 hybrid mice regressed MH134 cells. The tumor-resistance of DDD mice could be transferred to F1 hybrid; however, tumor-sensitivity of F1 hybrid could not be transferred to DDD mice. The fact that transfer of tumor-resistance is strengthened by preoperative irradiation of ^{60}Co to F1 hybrids suggests an important role of cellular factors in such a resistance.

DISCUSSION

Parabiosis between mice strains differing at the H-2 locus induces spontaneous separation of parabiotic partners or death due to so-called „parabiotic disease“ about the second week of union and only a few animals could survive for over 3 weeks. Several previous communications described that the death of both partners by parabiotic intoxication occurred at the same time. However, thoroughness of postoperative observation showed us that death of one partner was followed by that of the other. Death of the second partner seems to be the lethal effects of the dead partner on the living one (11). Parabiotic intoxication is commonly observed in F1 hybrid after parabiosing parental animals and their hybrid (16,17).

There are two techniques of parabiosis. In the first method, only skin flaps and abdominal muscle are sutured without making communication between the peritoneal cavities. In the second technique, on the contrary, the peritoneal cavities of both partners are communicated. In our experiments we have employed the latter method. The duration of parabiotic

union seems to be slightly prolonged by using the first technique. This may be due to an extent of communication of humoral or cellular factors between parabionts. Although *Ranzi* (26) first reported an existence of „parabiotic disease“ in 1909, its true mechanism has been unknown for a long period. In 1962 *Nakic* (23) initially demonstrated that destruction of the lymphatic tissue occurred following competition of immunological cells through the vascular anastomosis between parabionts. *Nakic* also described that parabiotic disease is very similar to graft-versus host reaction. *Hall & Hall* (15) have observed the growth of the capillary shunt between the parabiotic partners. *Cornelius* and his co-workers (10) explained that anemia or leucopenia of the hybrid would be the result of blood shunt formation from an F₁ hybrid to its parental partner. As shown by *Eichwald* and his associates (11), parabiotic intoxication was observed in F₁ hybrid-to-parental animal parabiosis following separation of union within 5 days, postoperatively.

We have investigated the above-mentioned problems by making use of of parabiotic technique between F₁ hybrid and its parents. In F₁ hybrid-to-C₃H/He parabiosis, many dead F₁ hybrid mice showed symptoms such as generalized weakness, loss of appetite, dermatitis, diarrhea, and alopecia from the end of the first week to the second week of parabiosis. White blood cells in the peripheral blood of such F₁ hybrid mice showed a rapid decrease on the second postoperative day and this was followed by a rapid rise on the fourth postoperative day. Then, a gradual fall of white blood cells occurred. Erythrocytes in such F₁ hybrid partners showed a gradual depression and in accordance with this, the skin of F₁ hybrid partners changed its color to pale. While the color of parental partner's skin seemed to be rather hyperemic than F₁ hybrid. In C₃H/He partner, leucocyte count showed a gradual decrease, however, the erythrocyte count kept constant levels. The serum electrophoretic pattern on the eighth day showed an increase of alpha- and beta-globulin and decrease of gamma-globulin in F₁ hybrid mice. Partner C₃H/

He mice, on the contrary, showed increase of albumin and decrease of alpha- and gamma-globulin. It is difficult to clarify the significance of such electrophoretic changes. As shown by *Nakic* and his co-workers(24), when the same mechanism is considered in parabiotic disease, the mice with this disease should always have splenomegaly (28, 30). However,, in our experiments, we did not always observe a constant tendency of splenomegaly. F1 hybrid mice irradiated with 500 r of ^{60}Co were joined in parabiosis with DDD mice. The spleen weight of such F1 hybrid mice was twice the control values.

When F1 hybrid mice irradiated with 500 r of ^{60}Co were parabiosed in the same way with C3H/He mice, the spleen weight of the F1 hybrid mice showed lesser value than the control. In comparison F1 hybrid-to DDD parabiosis with showed more rapid appearance of parabiotic intoxication and early death than F1-to-C3H/He parabiosis. From these results, it may be considered that chronic parabiotic disease does not always develop splenomegaly because of rapid destruction of the lymphatic tissue. Both parabiotic disease and homologous disease undoubtedly induce significant atrophic changes in the lymphatic tissue of the diseased animals, but there was no particular change in other organs (2, 29).

In parabiosis between F1 hybrid mice and DDD mice full thickness skin grafts from DDD parabionts to F1 partners and from F1 to DDD did not induce separation of the parabiotic union. Although the skin grafts from DDD to F1 hybrid mice survived for a long period, those from F1 hybrid to DDD mice were rejected after 8.7 days on an average. As the skin grafts from free (non parabiosed) F1 hybrid mice to DDD mice survived for 37.2 days, it is possible to say that DDD-parabionts were immunized by F1-parabionts. We considered that the second set phenomenon was induced by cellular or humoral exchange following parabiosis. Skin grafts from DDD mice to C3H/He mice irradiated with 600 r of ^{60}Co showed a survival time of 9-26 days (average: 18.5

days) which is significantly greater than that seen in controls (11.2 days).

F₁ hybrid mice irradiated with 600 r of ⁶⁰Co were parabiosed with C₃H/He mice. Such parabiosed C₃H/He mice kept the skin grafts from DDD mice for over 60 days. These results suggest that the tolerant condition of parental animals for F₁ skin grafts following F₁-to-parent parabiosis might be induced by irradiation of the host animals. After sensitizing DDD mice with sheep erythrocytes, parabiosis was established between such sensitized DDD and F₁ hybrid mice. It appeared that hemolysin antibody was passively transferred to the F₁ hybrid. In sensitized F₁ hybrid-to DDD parabiosis, the hemolysin titer of DDD showed a slight increase on day 9, but its level was distinctly lower than in the F₁ hybrid. The immunological reaction of the DDD against the F₁ hybrid partner seemed to disturb transfer of antibody from the F₁ hybrid to its parent DDD. The same phenomenon was observed after maintaining parabiotic union for 10 days. However, a rapid increase of hemolysin titer occurred following separation of the parabiotic union between the sensitized DDD and F₁ hybrid. This suggests an increase of cells antigenically stimulated with sheep erythrocytes in the F₁ hybrid mice. When these F₁ hybrid mice were treated with a booster dose of sheep erythrocytes, all of them died because of a hypersensitivity reaction. The complement level of DDD mice (below 20) was significantly lower than that of F₁ hybrid mice (average: 74.5). The complement level in F₁-to-DDD parabionts showed no significant change within one week after parabiosis. However, the complement level of DDD showed a rapid increase, and 4 weeks after parabiosis the complement titer of DDD mice and their partners were at almost the same level. This result seems to contradict the experimental data of transfer of sheep antibody. Complement titer of the DDD partner 2 days following parabiotic separation showed no significant change as compared with the preoperative level. The increasing factors of complement in DDD mice thus seemed to show a rapid disappearance following separation

of parabiotic union. C₃H/He mice irradiated 600 r of ⁶⁰Co showed decrease of complement level.

When normal bone marrow cells (above 2.0×10^4 cells) from isologous mice were intravenously injected for 3 times, the complement level increased. The degree of increase in complement level ran parallel with the amount of cells injected. The complement titer did not increase after injection of serum or bone marrow cells treated ultrasonically. This suggests that an increase of complement level in DDD mice following parabiosis seemed at least to be influenced by cellular factors (18). It is reasonable to consider that the cells of F₁ concerned with fluctuation of complement titer in DDD mice would be rejected immediately by the immunological reaction of DDD.

The mechanism of anemia in F₁ hybrid mice with parabiotic disease has been discussed by various investigators (11). Some authors described that red blood cells of F₁ hybrid transmigrate to its parabiotic partner following recovery of blood circulation between both partners. When this occurred red blood cell agglutination caused by the immune response of the partner was observed whereas the red blood cells of partner mice could not be agglutinated after migrating to the F₁ hybrid. As agglutinated erythrocytes were unable to return to the hybrid this would result in anemia. Another explanation is that vascular growth from each partner into the other permits both inflow and outflow of blood to a limited area of the partner. In consequence, blood from one partner could circulate through a limited area of the other without utilizing the other partner's blood vessels. In the pure partner the immune response would eventually obliterate both the direct vascular anastomoses and the hybrid vessels. But the hybrid, being unable to obliterate the blood vessels of the pure partner, would remain exposed to its immune response though the direct vascular anastomoses between the partners were already obliterated. The anemia of the hybrid mice, would then be the consequence of the immune response on the hybrid partner.

Both explanations offered fail to take into account that anemia in the hybrid tends to progress after separation of the parabiotic union. It has been reported that animals can recover from parabiotic disease after separation of the parabiotic union. At that time, the duration of parabiotic union should be kept in mind. It is unlikely that parental antibodies having crossed over into the hybrid prior to separation of union would make themselves increasingly felt during the week thereafter. However, it is possible that the immune effect would become manifest through parental cells which have crossed over into the hybrid prior to the separation of union.

Various investigators have had various opinions about the possibility of transfer of tumor-resistance from the host to the parabiotic partner. We have observed that anti-tumor factors of the pure partner could be transferred to the hybrid partner.

With 200×10^4 cells of MH₁₃₄ ascitic tumor cells originating from C₃H/He mice and F₁ hybrids survival time of such MH₁₃₄-bearing C₃H/He mice and hybrids was 23.0 days and 28.3 days, respectively. However, all of the DDD mice showed regression of MH₁₃₄ tumor cells. The separation of C₃H/He-to-F₁ hybrid parabiosis was performed 10 days after parabiosis and MH₁₃₄ tumor cells were inoculated to both partners one week following separation. Mean survival time of these two separated partners was 31.5 days in F₁ hybrid and 25.1 days in C₃H/He mice.

No significant difference was observed in comparison with a control group. F₁ hybrid-to-DDD parabiosis was separated after 20 days and MH₁₃₄ tumor cells were inoculated to these separated partners. Although the DDD mice showed no significant difference in tumor-regression as compared with the control group, 35 per cent of the F₁ hybrid mice showed regression of the MH₁₃₄ tumor. This suggests the possibility that anti-tumor factors of DDD mice were transferred by the method of parabiosis. F₁ hybrid mice irradiated

with 500 r of ^{60}Co and normal DDD mice were separated 10 days after parabiosis. 10 days after separation MH134 tumor cells were inoculated. 40 per cent of the F₁ hybrid mice showed regression of the tumor. Possibly the transfer effect of anti-tumor factors is accelerated by irradiation of F₁ hybrid mice. There is a strong probability that cellular factors play an important role in the development of anti-tumor effects. In addition transfer of parental cells to the F₁ hybrid mice should be accelerated.

It has been reported that parabiotic union between homologous animals induced transfer of tolerance for tumor (2). The immune tolerance produced by parabiosis is considered to be effectuated by tolerant donor cells fixed in the recipients. Genetic relationship between donor and recipient holds the key to the transfer of immune tolerance. When one partner has a H-2 allele not carried by the other as in F₁ hybrid-to-parent unions, immune tolerant factors are transferred from the parent to the F₁ hybrid. On the contrary it is not easily transferred from F₁ hybrid to parent.

SUMMARY

1. The duration of parabiotic union was examined in adult mice. Parabiotic union between histoincompatible animals showed spontaneous separation within 1-2 weeks. Sometimes one parabiotic partner died of parabiotic disease in such a histoincompatible union.

On the contrary, parabiotic union between isologous strains continued for over 180 days.

2. In parabiosis between (DDD ♀ × C₃H/He ♂) F₁ hybrid and parental C₃H/He mice, the duration of union was almost the same as in other histoincompatible parabioses. However, the union showed a duration of 10-100 days in (DDD ♀ × C₃H/He ♂) F₁ hybrid-to-DDD parabiosis. In such a combination, parabiotic disease exclusively developed in the F₁ hybrid partner which died within 2-3 weeks because of anemia, loss of body weight, diarrhea and atrophy of the lymphatic tissue.

3. F₁ hybrid mice irradiated with 500 r of ⁶⁰Co were joined in parabiosis with C₃H/He or DDD mice. One week following this, the spleen weight of these parabionts was measured. The spleen weight of non parabiosed F₁ (only irradiated ⁶⁰Co) was 0.078 gm on an average.

In irradiated F₁ hybrid-to-non irradiated DDD parabiosis, the F₁ hybrid parabiont showed a spleen weight of 0.158 gm and the DDD parabiont of 0.296 gm. In irradiated F₁ hybrid-to-C₃H/He parabiosis, the spleen weight of the F₁ hybrid parabiont was 0.054 gm and that of the C₃H/He was 0.144 gm.

4. In F₁ hybrid-to-DDD parabiosis, skin grafts from DDD mice to both partners survived for 10–100 days. Skin grafts from C₃H/He to F₁ hybrids were accepted. However, grafts from C₃H/He to DDD were rejected at 14.8 days on an average (9–27 days). When skin grafts were exchanged between both parabiotic partners, F₁ hybrids accepted the skin graft from DDD and DDD rejected the skin from F₁ hybrids at 8.7 days on an average (7–16 days). Mean survival time of skin grafts from non parabiosed F₁ hybrids to DDD was 37.2 days. Such a shortening of skin survival time must be due to an immune response.

5. 600 r ⁶⁰Co irradiated C₃H/He mice and F₁ hybrid mice were joined in parabiosis. Immediately after parabiosis, skin grafting from DDD mice was performed. 40 per cent of the skin grafts survived for over 60 days.

6. When DDD mice sensitized with sheep erythrocytes were parabiosed with non sensitized F₁ hybrid mice. The two partners showed almost the same hemolysin titer. When F₁ hybrids sensitized with sheep erythrocytes were joined in parabiosis with non sensitized DDD mice, the hemolysin titer of the DDD parabionts was significantly lower than that of the F₁ hybrid partners.

7. The complement level was examined in F₁ hybrid-to-DDD parabiosis. The complement level of the DDD partner was below 20 until 6 days following parabiosis. One week after parabiosis it returned to almost the same level as that

of the F₁ hybrid. Two weeks after parabiosis between F₁ hybrids and DDD the parabiotic union was separated. The complement levels of the separated DDD partners showed a rapid decrease 2 days after the separation.

8. After irradiation of 800 r ⁶⁰Co, the complement level of C₃H/He mice showed a decrease. The complement titer rapidly recovered following injection of 9.0×10^7 of isologous bone marrow cells. However, the complement level did not show recovery by inoculation of ultrasonically treated bone marrow cells or by injection of normal serum.

9. MH₁₃₄ tumor cells originating from C₃H/He mice were subcutaneously inoculated to C₃H/He and (DDD ♀ × C₃H/He ♂) F₁ hybrid mice.

The MH₁₃₄-bearing C₃H/He and the F₁ hybrids died of tumor 23.0 days and 28.3 days after inoculation, respectively, while 100 per cent of MH₁₃₄-bearing DDD showed regression of the tumor. F₁ hybrids and C₃H/He mice were parabiosed for 10 days and then separated. One week after the separation MH₁₃₄ tumor cells were inoculated to both partners.

The MH₁₃₄-bearing F₁ hybrid partners and C₃H/He partners died 37.5 days and 25.1 days, respectively, because of tumor. F₁ hybrid-to-DDD parabiosis was maintained for a period of 20 days and then separated. One week after the separation, MH₁₃₄ tumor cells were transplanted. 35 per cent of MH₁₃₄-bearing F₁ partners showed regression of the tumor. F₁ hybrid mice irradiated with 500 r ⁶⁰Co were joined in parabiosis with DDD mice for 10 days and then separated. 40 per cent of the separated F₁ hybrid mice demonstrated tumor regression.

From these results it may be concluded that recolonization of cells occurs from parental animals to F₁ hybrid animals and the cells transmitted from F₁ hybrids to parental animals are rejected by the immune response of the hosts. Thus, a true cellular chimera is not established.

ACKNOWLEDGEMENT

We wish to thank Professor Yukio Ishibashi for helpful advice.

REFERENCES

1. *Aelber, G.*: Zur Frage der Übertragbarkeit natürlich Tumorresistenz durch Parabiose. Zeitschr. für Krebsforschung 68, 150, 1966.
2. *Aust, J. B., Rogner, W. & Guttman, R.*: The antigen persistence concept of homograft tolerance: Immunologic response to tumor and spleen cells following transfer, reimplantation, and parabiosis. Ann. Surgery 162, 738, 1965.
3. *Bichel, J. & Holm-Jensen, I.*: Parabiosis and resistance to transplantation. III. Studies on resistance to transmissible leukemia in mice by means of heterogenetic and homogenetic parabiosis. Acta Path. et Microbiol. Scand. 26, 319, 1949.
4. *Bichel, J. & Holm-Jensen, I.*: Determination by means of radioactive blood corpuscles of the crossed blood flow between parabiotic mice. Acta Physiol. Scand. 17, 255, 1949.
5. *Billingham, R. E., Brent, L. & Medawar, P. B.*: Acquired tolerance of tissue homografts. Ann. N.Y. Acad. Sci. 50, 409, 1955.
6. *Billingham, R. E. & Medawar, P. B.*: The technique of free skin graftings in mammals. J. Exp. Biol. 28, 385, 1951.
7. *Bunster, E. & Meyer, R. K.*: An improved method of parabiosis. Anat. Res. 57, 339, 1933.
8. *Cauchi, M. N.*: Fixation of parental strain lymphocytes in F₁ hybrid rats during cross-circulation. Nature 209, 817, 1966.
9. *Cloudman, A. M.*: Reaction of hybrid and parabiotic pseudo-hybrid mice to inoculations of tumor C 193. Cancer Res. 3, 47, 1943.
10. *Cornelius, E. A. & Martinez, C.*: Transfusion experiments and survival studies in parabiosis intoxication. Proc. Soc. Exp. Biol. Med. 120, 426, 1965.
11. *Eichwald, E. J., Lustgraaf, E. C. & Strainer, M.*: Genetic factors in parabiosis. J. Nat. Cancer Inst. 23, 1193, 1959.
12. *Falls, N. G. & Kirschbaum, A.*: Passive immunization of mice in parabiosis against a transplanted lymphosarcoma. Cancer Res. 13, 741, 1953.
13. *Fujii, G. & Goto, S.*: Determination of complement level in mice: Changes of complement level in iso- and homograft reactions. Jap. J. Allergy 14, 33, 1965.
14. *Furth, O. B., Barnes, H. A. & Broxner, A. B.*: Studies on resistance to transmissible leukemia in means of parabiosis. Arch. Pathol. 20, 163, 1940.
15. *Hall, C. E. & Hall, D.*: On the nature of parabiosis intoxication: Shock as the precipitating cause. J. Exp. Med. 103, 263, 1959.

16. Hilgard, H. R., Cornelius, E. A., Dalmaso, A. P., Martinez, C. & Good, R. A.: Immune mechanisms in parabiosis intoxication. *J. Exp. Med.* 119, 567, 1964.
17. Jensen, E. & Simonsen, M.: Part IV. Experimental induction of altered reactivity to tissue transplants. Induced tolerance after parabiosis. Apparent facilitation of tolerance by a simultaneous graft-versus-host reaction. *Ann. N.Y. Acad. Sci.* 99, 657, 1962.
18. Kuroiwa, T.: Studies of changes in complement level by bone marrow transplantation. *Transpl. J* 11, 1, 113, 1967. (Japanese).
19. Martinez, C., Smith, J. M., Aust, J. B. & Good, R. A.: Transfer of acquired tolerance to skin homografts in mice. *Proc. Soc. Exp. Biol. Med.* 98, 640, 1958.
20. Morgan, H.: The growth of animal tumors in parabiotic mice. *Cancer Res.* 3, 546, 1943.
21. Mori, G. & Kobayashi, M.: Practice in electrophoresis, Nankodo, Tokyo, 1956.
22. Nakic, B. & Silobrcic, F.: Tolerance of skin homografts related to fatal disease in separated rat parabionts. *Nature* 182, 264, 1958.
23. Nakic, B.: The concept of competitive replacement. *Ann. N.Y. Acad. Sci.* 90, 689, 1962.
24. Nakic, B., Nakic, Z. & Silobrcic, F.: Graft-versus-host reaction in parabiotic disease. *Nature* 186, 322, 1960.
25. Owen, R. D.: Immunogenetic consequences of vascular anastomosis between bovine twins. *Science* 102, 400, 1945.
26. Ranzi, E. & Ehrlich, H.: Über die Wirkung von Toxinen und die Bildung von Antikörpern bei parabiotischen Tieren. *Zeitschr. Immunitätsforsch. und Exp. Therap.* 3, 38, 1909.
27. Sauerbruch, F. & Heyde, M.: Über Parabiose künstlich vereinigter Warmbluter, *Münch. Med. Wochenschr.* 55, 153, 1908.
28. Schwarz, R. S. & Beldoff, L.: Homologous disease reactivation by x-radiation. *Science* 140, 171, 1963.
29. Silobrcic, F. & Trentin, J. J.: On the mechanism of „Homologous Disease“ in sublethally irradiated mice. *Transplantation* 4, 719, 1966.
30. Zeiss, I. M. & Fox, M.: Donor and host contribution to splenomegaly in homologous mouse chimerism. *Nature* 197, 673, 1963.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.