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Tolerance of F_1 Hybrid Skin Homografts in the Parent Strain Induced by Parabiosis.* (25483)

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Immunological tolerance allowing homotransplantation of tissues and organs has been achieved by exposing animals to donor strain antigens prior to delivery or during immediate neonatal period(1). However, our recent reports indicate that weanling Z mice, injected with viable spleen cells taken from (Z x Ce) F_1 hybrid individuals, became tolerant and accepted skin grafts from (Z x Ce)F_1 donors (2). Similarly, older female mice of A and C_{57}Bl (Subline 1) strains were made tolerant of male skin isografts either by intravenous injection of male spleen cells or by establishing a parabiotic union of adult females with isologous males(3). Further, using the technic of parabiosis, it has also been demonstrated that tolerance of homologous tissue transplants, induced during neonatal period, can be transferred to normal adult mice of the same strain. In the latter experiment, the previously non-treated parabiont lost its ability to reject homologous tissue and, like its partner, accepted the homologous skin graft (4). Therefore, it seems that under certain well-defined conditions, induction of immunological tolerance in mice may not be limited to antigen exposure during embryonal life or immediately after birth. Our incidental observations indicate that, contrary to expectation, parabiosis performed between certain F_1 hybrid mice and individuals of either parent strain were successful in most instances and resulted in induction of tolerance in the parent parabiont whereby this animal lost its capacity to recognize and reject homologous skin grafts taken from hybrid donors of the corresponding F_1 cross. It is the purpose to document and further extend these observations.

Method. Highly inbred mice of the Z§, Ce, A, C_{57}Bl (Subline 1) and their reciprocal F_1 hybrids were used. In one group of experiments parabiosis was established between (Z x Ce)F_1 hybrids and Z animals, and between (Z x Ce)F_1 and Ce. In this group mice were from 40 to 70 days of age at time of parabiosis. In another group parabiosis was performed between (A x Z)F_1 and Z, and between (A x Z)F_1 and A mice. These animals were from 30 to 46 days old when placed in parabiosis. In a third group (Z x C_{57}Bl)F_1 and Z, and (Z x C_{57}Bl)F_1 and C_{57}Bl mice were similarly united in parabiosis. These animals were from 30 to 50 days old when parabiosis was established. In all instances parabiotic animals were of the same sex. Each preceding group had its own controls, consisting of parabiotic pairs of either parental strain, *i.e.*, Z to Z and Ce to Ce for the first group, Z to Z and A to A for the second, and C_{57}Bl to C_{57}Bl and Z to Z for the third group. Parabiosis was of the celomic type(5-6), consisting essentially of surgical union of animals through midlateral incisions comprising skin, muscle and peritoneal cavities. Parabiotic pairs were housed individually in plastic mouse cages with free access to Purina Fox Chow and tap water. Parabiosis was maintained from 20 to 45 days, after

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§ Z is used to simplify designation of mice of the C_3H strain, originally obtained in 1956 from Dr. John J. Bittner's mouse colony.

TABLE I. Immunological Tolerance of Homologous F_1 Hybrid Skin Grafts Induced in Mice of Their Parent Strains by Way of "F_1 to Parent" Parabiosis.

Parabiosis groups		No. of pairs	Age when joined (days)	Time in parabiosis (days)	No. of pairs dead*	Successful F_1 hybrid skin graft on parent parabiont†
(Z × Ce) F_1	Z	28	45–70	35	2	26/26‡
Idem	Ce	12	40–50	20	2	10/10
Z	Z	28	53–72	35	4	0/24
Ce	Ce	14	34	20	4	0/10
(A × Z) F_1	Z	16	36–46	45	3	12/13
Idem	A	15	30–35	22	12	0/3
Z	Z	16	35–45	25	3	0/13
A	A	14	25	20	4	0/10
(Z × C_{57}Bl) F_1	Z	14	35–45	45	4	2/10
Idem	C_{57}Bl	12	30–35	25	4	0/8
Z	Z	12	44	20	2	0/10
C_{57}Bl	C_{57}Bl	12	50	20	2	0/10

* Death occurred prior to skin grafting. † Grafts were performed 8 days after disruption of parabiosis. ‡ No. of successful grafts/No. grafted.

which parabiosis was surgically discontinued under nembutal anesthesia, closing each peritoneal cavity with 5-0 catgut sutures, and both muscle and skin layers with metal clips. Eight days after disruption of parabiosis, the parent member of the pair received a full-thickness skin graft taken from hybrid donor of corresponding F_1 cross. In all instances donor and recipients were of the same sex and approximately the same age. The technic for skin graft was as previously described(1). In most instances, mice were kept under observation for at least 5 months following skin transplantation. In evaluating success or failure of graft, the direction of the hair growth was of primary importance, since the method involves a 180° rotation of donor skin prior to implantation on recipient. Further, successful grafts were regularly noted to increase in size with growth of host.

Results. The results obtained are summarized in Table I. Of 28 parabiotic pairs of (Z x Ce)F_1 with Z that were kept in parabiosis for 35 days, 2 died prior to skin grafting. All remaining 26 parent Z strain parabionts were tolerant and accepted the (Z x Ce) F_1 skin graft. Similar results were obtained in Ce animals held in parabiosis with (Z x Ce)F_1 hybrids, since in all instances the Ce parent parabionts were tolerant of this hybrid skin. On the other hand, none of the Z to Z or Ce to Ce parabiosis control animals accepted the F_1 hybrid graft.

In the group of (A x Z)F_1 joined in parabiosis with Z animals, 3 of 16 died prior to skin grafting. Of the remaining 13 Z parabionts, 12 were tolerant and accepted skin grafts from (A x Z)F_1 hybrid donors. Control Z animals which had been in parabiosis with Z animals regularly rejected the (A x Z)F_1 skin. In contra-distinction, in group in which parabiosis was established between (A x Z)F_1 hybrid and A parent strain mice, 12 of 15 pairs died during parabiotic union, and none of the 3 remaining parent A strain parabionts accepted the F_1 skin graft. As in other studies, the controls for this group always rejected the F_1 skin graft. Finally, in the group in which parabiosis was established between (Z x C_{57}Bl)F_1 and Z animals, of a total of 14 pairs prepared, 4 died during parabiosis. Of the remaining 10 pairs, only 2 of the Z parabionts were tolerant and accepted the (Z x C_{57}Bl)F_1 hybrid skin graft. In the group in which parabiosis was established between (Z x C_{57}Bl)F_1 and the C_{57}Bl parent, 4 out of 12 pairs died during parabiotic union, and none of remaining eight C_{57}Bl partners accepted subsequent F_1 hybrid skin graft. Here again, control Z animals that were in parabiosis with isologous Z mice, and C_{57}Bl animals united to animals of the same strain, all rejected skin grafts taken from (Z x C_{57}Bl)F_1 hybrid individuals.

Discussion. The results seem to demonstrate that by joining in parabiosis F_1 hybrid mice resulting from the cross between 2 inbred strains of mice with animals of either of

their parent strains, it is sometimes possible to obtain immunological tolerance of F_1 skin homotransplantation in the parent parabiont. In the second place, they confirm our previous observations indicating that by this method tolerance can sometimes be induced in adult individuals(3). This is clearly seen when parabiosis was established between (Z x Ce)F_1 and individuals of either of their parent strains, *i.e.*, Z or Ce. Similar results were obtained in Z animals joined in parabiosis with (A x Z)F_1 hybrids. However, when these hybrids were joined in parabiosis with mice of the A parent strain, death occurred in 80% of parabionts, and in those surviving parabiosis, the A parent parabiont rejected the F_1 hybrid skin homograft in every instance. Thus, a real difference between A and Z animals with respect to susceptibility to induction of tolerance by this manipulation seems apparent.

When (Z x C_{57}Bl)F_1 mice were placed in parabiosis with Z animals, 29% of the animals died, and of the surviving Z strain parabionts, only 20% became tolerant and accepted the corresponding F_1 hybrid skin graft. Further, parabiosis established between this same hybrid and C_{57}Bl parent strain also led to death in approximately 30% of parabionts, and the procedure failed to induce tolerance in any of the C_{57}Bl mice surviving parabiotic union. It seems clear that, whereas immunological tolerance permitting subsequent homotransplantation of skin can be achieved in certain strains of mice as a result of placing mice in parabiosis with a member of an F_1 combination, members of the other parent strain similarly treated, will not become tolerant. This result is particularly clear when the effects of parabiosis between (A x Z)F_1 and Z are compared with those obtained with (A x Z)F_1 and A.

It appears that strain differences exist with respect to capacity both to induce and accept tolerance by the method of parabiosis. Interpretation of these results is difficult. The hypothesis previously advanced to explain induction of tolerance in weanling Z mice by intravenous injection of spleen cells taken from (Z x Ce)F_1 hybrids(2) and tolerance of male skin in females of the same strain after parabiosis(3), is attractive. According to this concept, weak histocompatibility differences are postulated to exist between members of these combinations, and cells capable of being conditioned are assumed to be present in the reticuloendothelial or lymphoid tissues throughout life. When the histocompatibility differences are slight, parabiosis can be established and tolerance is eventually accomplished as cells from the hybrid enter the body of the parent parabiont. Strong histocompatibility differences between parabionts would be expected to preclude such a result, since the parabiotic union would fail primarily as a consequence of immunological interaction between the partners.

The results obtained in the second group of experiments in which Z animals were made tolerant of (A x Z)F_1 tissue, whereas A mice did not become tolerant when joined in parabiosis with the same F_1 hybrid, are more difficult to explain. Since these strains differ at the H_2 genetic locus, this hypothesis provides no explanation for susceptibility of Z animals to development of tolerance and resistance of the A strain animal to this phenomenon. However, a possible explanation for such a discrepancy is that genic interaction, such as that postulated by Fox(7) might have occurred by crossing these 2 strains, and there is lesser histocompatibility difference between Z and the (A x Z)F_1 than exists between A and the (A x Z)F_1. Such an occurrence might make it easier to establish parabiosis between Z and (A x Z)F_1 and also easier to develop tolerance once parabiosis has been established.

The mechanism responsible for induction of tolerance by parabiosis is not known. In this regard it seems worthy of considering the similarity of this situation to that demonstrated to exist by Egdahl and Hume in cross-circulated dogs(8). These authors found that, during period of cross circulation with donor, dogs would not reject homotransplanted kidney, even though they had been previously immunized, whereas termination of cross circulation resulted in almost immediate evidence of rejection of the kidney homotransplant. Kamrin's(9) observation that parabiosis in the rat may permit prolonged survival of renal tissue homotransplants is

also pertinent. Whatever the ultimate explanation for these observations, it is clear that in appropriate strain combinations a form of immunological tolerance can be achieved by parabiosis in mature animals. Although strains of mice used and the explanation of the phenomenon were somewhat different than that provided here, Rubin(10) using small number of mice has made similar observations.

Summary. 1) Tolerance of F_1 hybrid skin homografts in mice of their corresponding parent strains has been attempted by establishing "F_1 to parent" parabiosis. 2) Results were: a) Mice of either Z or Ce strain joined in parabiosis with (Z x Ce) F_1 hybrids became tolerant and accepted skin homografts taken from this hybrid. b) Tolerance of (A x Z)F_1 skin was also obtained in Z strain mice following parabiosis with (A x Z)F_1 hybrids. However, A mice joined to the same hybrids failed to develop tolerance and rejected the F_1 grafts in all instances. c) While 20% of Z mice became tolerant of (Z x C_{57} Bl)F_1 skin after being in parabiosis with this hybrid, none of C_{57}Bl parent parabionts were susceptible of the hybrid skin grafts.

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Delayed Repolarization in Smooth Muscles. (25484)

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A delay in repolarization following spike depolarization gives a plateau in heart muscle normally(1), in squid giant axons after injection of tetraethyl amine(2), and in mylinated nerve in presence of barium(3). The plateau in heart muscle can be varied in duration by a variety of treatments and appears to depend largely on a delayed rise in potassium conductance(4). The plateau in frog ventricular muscle is lengthened by barium(5). A plateau-type potential normally occurs in the ureter(6) but is unlike that in heart in being prolonged by quaternary ammonium compounds and shortened by epinephrine and low calcium(7). In other smooth muscles the spike normally shows equal rates of depolarization and repolarization(8,9,10).

Experimental. Action potentials were recorded from cat intestinal muscle by means of the sucrose gap electrode(11). When 0.1% barium chloride was added to the perfusing Krebs solution, 45 mv. spikes appeared which were soon transformed into plateaus (Figs. 1 and 2). The duration was increased from a spike of about 100 msec to depolarizations which persisted for as long as 70 seconds. The effect was reversible, as shown by washing out the barium (Fig. 1-c, d). The longer plateaus were usually characterized by oscillations, especially just prior to their termination (Fig. 1-b, Fig. 2-a, b, c).

Plateaus with trains of spikes on their crests were characteristically produced in cat longitudinal intestinal muscle by acetylcholine (10^{-6}) (Fig. 3-a, b). A higher concentration (10^{-5}) produced maintained depolarizations accompanied by spike activity (Fig. 3-c) as described for taenia coli(12). A critical con-

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