comparable to those on media containing dialyzed serum. Suspension cultures of Walker carcinosarcoma 256 were grown with continuous passage in the serumless medium.

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Essential Duration of Parabiosis and Development of Tolerance to Skin Homografts in Mice.* (25798)

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Immunological tolerance of F_1 hybrid skin homografts can be induced in mice of their parent strains after establishing a "F_1 hybrid to parent" parabiosis(1,2). Similarly, female mice of either the A or C57Bl (Subline 1) strains were made tolerant of male skin isografts by placing adult females in parabiosis with the corresponding males(3). Finally, establishment of parabiosis between animals of the Z strain made tolerant of Ce strain tissue by intravenous injection of Ce cells at birth and previously non-conditioned isologous Z mice led to transfer of tolerance. Thus the Z strain animals conditioned only by having been placed in parabiosis with Z animals tolerant of Ce tissue lost their ability to reject homologous skin transplanted from Ce individuals(4). On the basis of these results we postulated that induction of tolerance by the technic of parabiosis might be due to a continuous exchange of all the transplantation antigens, perhaps in the form of intact cells between the 2 parabionts(2,3,4). However, recent experiments performed in this laboratory indicate that length of time during which parabiosis between homologous individuals must be maintained to achieve suppression of the homotransplantation reaction varied with strain of mice used, suggesting that time of foreign antigen exposure and exchange necessary to induce immunological tolerance in a given individual by this method, might perhaps be dependent on degree of histocompatibility difference between the individual and its parabiotic partner, *i.e.*, the more similar the 2 partners, the shorter the length of time in parabiosis required to achieve immunological tolerance.

Our purpose is to document observations bearing on this point and to demonstrate that duration of parabiosis required to produce tolerance is indeed a function of degree of histocompatibility difference between individuals and is also a characteristic of the strains of animals involved regardless of histocompatibility difference.

Method. In one group of experiments mice of the Z† strain and F_1 hybrids resulting from

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† Z is used to designate mice of C_3H strain originally obtained from Dr. J. J. Bittner's mouse colony.

TABLE I. Essential Duration of Parabiosis in Development of Tolerance to Skin Homografts in Mice.

Parabiosis	Time in parabiosis (days)	Time from separation to grafting (days)	No. of Z parabionts accepting AZF_1 skin grafts/total No. grafted
			%
Z-----AZF_1	7–10	6	1/9 (11)
Idem	23	13	2/7 (29)
"	30	5	7/8 (88)
"	30	20	9/9 (100)
"	40–45	5	23/25 (92)
Z-----Z	25	7	0/13 (0)

the cross between A females and Z males were used. When these animals were approximately 3 months old a "AZ F_1 to Z parent" parabiosis was established. Parabiosis performed was that of the celomic type, as previously described(3). Mice joined together were of approximately the same age and of the same sex. Nine, 23, 30 and 45 days following union, groups of parabiotic pairs were surgically separated under nembutal anesthesia. The peritoneal opening of each parabiont was closed with 5-0 catgut sutures and both muscle and skin layers were approximated with metal wound clips. At varying periods following disruption of the parabiotic union (Table I) the Z partners received a full-thickness abdominal skin graft taken from homologous (A x Z)F_1 hybrid donors. The technic of skin grafting as well as the criteria used to establish acceptance or rejection of the graft were the same as previously described(5). Following grafting of the skin, animals were observed for periods ranging from 5 to 10 months. In a second group parabiosis was established between mice of the Z and Ce strains when these animals were approximately 45 days old. After establishment of the parabiotic union these mice were divided into 2 experimental groups. The members of one group were separated after having been in parabiosis for 27 days and the members of the other group were maintained in parabiosis for a period of 64 days. Eight days following separation mice of both groups received homologous skin grafts taken from adult donors of the same strain as those of their respective partner. After skin grafting, mice were observed for periods ranging from 5 to 10 months to evaluate skin graft survival. For both experiments proper controls consisting of pairs of isologous mice were prepared. After having been kept in a state of parabiosis for periods ranging from 20 to 35 days, members of the control parabiotic pairs were separated and each parabiont was grafted with the corresponding homologous skin, *i.e.*, Z with either (A x Z)F_1 or Ce and Ce with Z.

Results. The results of the first experiment are recorded in Table I. Of 9 Z strain mice which had been in parabiosis with (A x Z)F_1 hybrids for a period of 9 days only, one became tolerant and accepted the (A x Z)F_1 hybrid homologous skin. Of 7 pairs in which parabiosis was discontinued at 23 days, 2 of the Z partners accepted the F_1 hybrid skin homograft. However, when parabiosis was allowed to continue for 30 days, 16 of 17 of the Z partners were found to tolerate (A x Z)F_1 skin grafts. Similar uniformly successful homografts were obtained in the groups maintained in parabiosis for 45 days prior to separation. It will be noted in the Table that time elapsing between disruption of parabiosis and skin grafting did not affect appreciably the results obtained in the different groups.

Table II shows results of second experiment. When parabiosis between Z and Ce mice was disrupted 27 days after establishment, 11 of 12 of the Z animals were tolerant of Ce skin homografts. In contradistinction none of the Ce animals which had been parabiotic partners of the same Z mice accepted homologous Z strain skin grafts. However, when the parabiotic union between Z and Ce animals was disrupted after 64 days tolerance

TABLE II. Essential Duration of Inter-strain Parabiosis on Development of Immunological Tolerance.

Parabiosis	Time in parabiosis (days)	Successful skin homografts*	
		Z partner	Ce partner
Z-----Ce	27	11/12	0/14
Idem	64	11/13	8/13
Z-----Z	35	0/24	
Ce-----Ce	20–40		0/10

* No. of takes/No. of grafted.

of Z skin was present in 8 of 13 Ce parabionts and tolerance of Ce skin was demonstrated in 11 of 13 of the Z parabionts.

In no instance did isologous parabiosis between individuals of either Z or Ce strain result in tolerance of the homologous skin grafts.

Discussion. The results of these 2 experiments are of interest from several points of view. They confirm previous reports demonstrating that acquired tolerance of F_1 hybrid homologous skin grafts can be induced in mice of the parent strain by establishing a "parent to hybrid" parabiosis(1,2). Perhaps more significant is the observation that a high degree of mutual tolerance of skin homografts can be achieved in mice of the Z and Ce strains when joined by this surgical procedure. These results extend to 2 different strains the observations of Rubin(1) that tolerance can be induced between mice of different inbred strains during adult life by placing them in parabiosis.

Results obtained in both experiments indicate that duration of the parabiotic union plays a critical role in determining whether or not tolerance will be induced. For example in the case of Z-----AZ F_1 union, disruption of parabiosis 23 days following operation permitted establishment of tolerance of the AZ F_1 hybrid skin homograft in only 3 of 16 (19%) of Z parabionts. However, when disruption of union was performed 30-45 days after establishment of parabiosis 39 of 42 (93%) of Z parabionts tolerated the corresponding F_1 hybrid skin homografts. Similar findings were obtained in the case of parabiosis between Z and Ce animals. In this instance, the results demonstrate that while 27 days of union was sufficient to abrogate the immunological capacity of most Z parabionts to reject the Ce strain skin graft, this length of time was not sufficient to condition the Ce parabionts to accept Z strain skin homografts. In contradistinction, after 67 days in parabiosis both members of the parabiotic pair became tolerant of the strain of the corresponding parabiont and accepted skin homografts from appropriate donors in almost every instance.

Although difficult of interpretation, as are all findings related to production of a state of tolerance in adult animals, our results add substantially to the accumulating evidence that under certain well defined conditions immunological tolerance can be produced in adult animals. In this regard the procedure of celomic parabiosis seems particularly opportune. The observations support the contention previously submitted that induction of tolerance by parabiosis might be due to a continuous exchange of transplantation antigens between both members of the pair in such amounts as to inhibit capacity for a homograft reaction. This state of non-reactivity which might be considered akin to a state of immunological paralysis could then permit establishment of cells containing all the transplantation antigens in a form capable of replication in the respective hosts, so that upon separation from the parabiotic union, these cells in residence within the recipient host, could maintain the tolerant state between the 2 strains.

It is of interest with respect to the mechanism involved that duration of the parabiotic union appears to be of critical significance. Our observations together with those of Mariani *et al.*(3) indicate that it is more difficult to establish tolerance between Z and AZ F_1 animals than between male and female animals of either the A or C57 Bl (Subline 1) strains by the technic of parabiosis. This conclusion is drawn since it took 30-45 days of parabiotic union to establish tolerance in the Z----AZ F_1 relationship and no more than 5-10 days of parabiosis to establish tolerance between males and females within the same strain.

The studies employing the relatively closely related Z and Ce strain animals (Rubin, personal communication) establish another point. Different strains of mice have different susceptibility to development of acquired tolerance during adult life. For example, in these experiments the Z animals were regularly made tolerant by remaining only 27 days in parabiosis with Ce individuals, whereas the Ce animals required a period of more than 60

days before tolerance was uniformly induced from the same parabiotic union.

Whatever the final explanation, it seems clear that immunological tolerance can be induced in adult animals. It also seems clear that the required antigenic dosage for development of tolerance is very critical and thus the length of time during which homologous parabiosis need be maintained to induce tolerance may be a function of this dosage, which in turn is at least partially consequent to degree of histocompatibility difference between strains. At any rate, length of time during which parabiosis must be maintained to induce immunological tolerance depends upon the strains of mice joined in parabiosis. This observation is strikingly similar to previous observations indicating differences among strains of mice as to capacity of developing classical immunological tolerance when splenic cells are injected intravenously during the neonatal period as well as differences in capacity of donor cells from various strains to induce this phenomenon(5).

Summary. 1. Parabiosis between Z animals and AZ F_1 hybrid animals results in immunological tolerance of the former strain to the latter. 2. When parabiotic union between Z and AZ F_1 animals is maintained less than 30 days a tolerant state is not regularly produced, but when maintained 30 days or more, tolerance of Z for AZ F_1 hybrid skin grafts is the rule. 3. Tolerance between Z and Ce strain mice may be produced in adult life by maintenance of a parabiotic union between members of these 2 strains. 4. Under these circumstances Z mice more readily become tolerant to the strain of the parabiotic partner than do Ce strain mice. 5. Implications of these findings to the theory of immunological tolerance are discussed.

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Penetration of Radioactive Phosphorus into Normal and Shocked Rats' Brain.* (25799)

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Traumatic shock is accompanied by changes in enzyme activities of liver, heart and brain. Strawitz and Hift(1) and Packer, Michaelis and Martin(2) have demonstrated uncoupling of oxidative phosphorylation, as well as shifts in concentrations of phosphate, sodium, potassium and calcium, accompanied by swelling of mitochondria in shock. The intracellular changes suggested that the permeability of some tissues might change in shock, and that in particular the blood brain barrier might be affected. This possibility was tested with an autoradiographic method. When the Masson trichrome stain, as modified by Goldner, was used on brains of normal and shocked rats‡ no significant differences could be found.

Method. Young adult Wistar rats weighing 200-300 g were injected intraperitoneally with P^{32} inorganic phosphate, approximately 35 μc per 100 g body weight. Shock was induced within 10 minutes after injection with the drumming method(3) in an apparatus consisting of 4 drums driven by one motor.

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