Effect of the Amount of Tissue Grafted Upon Survival of Skin Homografts.* (26374)

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The effect of the dose or amount of tissue transplanted into homologous hosts upon graft survival has been the subject of several investigations. Medawar(1,2) had previously demonstrated that mean survival time of skin homografts performed in rabbits varies inversely with quantity of tissue grafted, *i.e.*, the smaller the graft the longer the time required for its rejection. These findings were confirmed in rats by Lehrfeld and Taylor(3) and more recently by Zoticov and Budik(4). However, the latter investigators also reported that transplantation of homologous skin in rats in which amount of tissue transplanted was larger than 25 cm^2 was well tolerated and survived for a long time, in some instances up to 8 months following transplantation. The prolonged rejection of large skin homografts observed was interpreted by these workers as the result of the effect of massive dose of foreign antigen contained in the graft leading to a suppression of the immunological capacity of the host.

Stimulated by these findings and because of certain theoretical considerations we decided to reinvestigate this problem determining survival time of male skin isografts of different sizes when placed on isologous female mice known to exhibit graft rejection based on the sex-linked histoincompatibility factor (5). Similar experiments were also performed using several homologous strain of mice. Specifically, this communication reports our observations on this subject and demonstrates that at least in female mice of the C57Bl (Subline 1) strain transplanted with male skin isografts the dose or amount of tissue grafted may be a determining factor in rejection or acceptance of such grafts.

Method. In one set of experiments, survival of male skin isografts of 2 different sizes when transplanted onto female mice of the C57Bl (Subline 1) strain was studied. One group consisting of 19 female mice of approximately 3 months of age received a 3 cm^2 (1.5 $\times$ 2.0 cm) full-thickness abdominal skin isograft taken from male donors of approximately the same age. The technic of skin grafting has been described(6). Another group of 12 females of similar age received a full-thickness male skin isograft of approximately 24.5 cm^2 (3.5 $\times$ 7.0 cm). The piece of skin to be transplanted was removed from the donor by performing 2 circular incisions comprising skin and subcutaneous tissue, one at the level of the axillae and the other at the level of the iliac crest. After careful dissection of the skin from the adjacent subcutaneous tissue and fat, the tube-shaped graft was pulled apart *in toto* over the head. In placing the graft on the host the skin tube was turned 180° and slipped on over the head of the recipient previously submitted to removal of a similar amount of skin. Interrupted 5-0 silk sutures were used to bring the skin transplant in a close union with the corresponding host tissues. No bandage or dressing was used.

Turning of the graft helped in determining the success or failure of the transplant since the hair in a succesful graft grows in an opposite direction to that on the skin of the host.

Similar experiments were performed using mice of homologous strains in the following donor-host strain combinations, Balb/C $\rightarrow$ DBA (Subline 2), DBA (Subline 2) $\rightarrow$ Balb/C and (A $\times$ Z) $F_1 \rightarrow$ Z.

Following surgery mice were singly housed in plastic mouse cases and fed Purina Laboratory Chow and tap water. Macroscopic inspections of the grafts were performed daily for a period of no less than 5 months.

Results. The results are summarized in

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TABLE I. Effect of Amount of Tissue Grafted upon Survival of Skin Homografts and Isografts.

Host strain	Donor strain	Graft size (cm^2)	No. of animals	No. and (%) of animals accepting skin grafts
C57Bl (Sub. 1) ♀	C57Bl (Sub. 1) ♂	3	19	4 (21)
		24.5	12	10 (83)
Balb/C	DBA (Sub. 2)	3	16	0 (0)
		24.5	10	0 (0)
DBA (Sub. 2)	Balb/C	3	20	0 (0)
		24.5	10	0 (0)
Z (C3H)	(A × Z) F_1	3	42	0 (0)
		24.5	6*	—

* All 6 mice died 3 to 5 days after grafting (see text).

Table I. In the group of C57Bl females transplanted with male skin isografts of 3 cm^2 in size, incidence of successful graft acceptance was 21% (4 out of 19). On the other hand, in similar females receiving large male skin isografts (24.5 cm^2) incidence of successful takes was 83% (10 out of 12) (Fig. 1).

In the groups of mice of the Balb/C strain receiving either small or large homologous skin grafts taken from DBA (Subline 2) donors all rejected the skin transplanted. Similar negative results were obtained when DBA (Subline 2) mice were used as recipients and Balb/C as donors regardless of size of the skin transplanted.

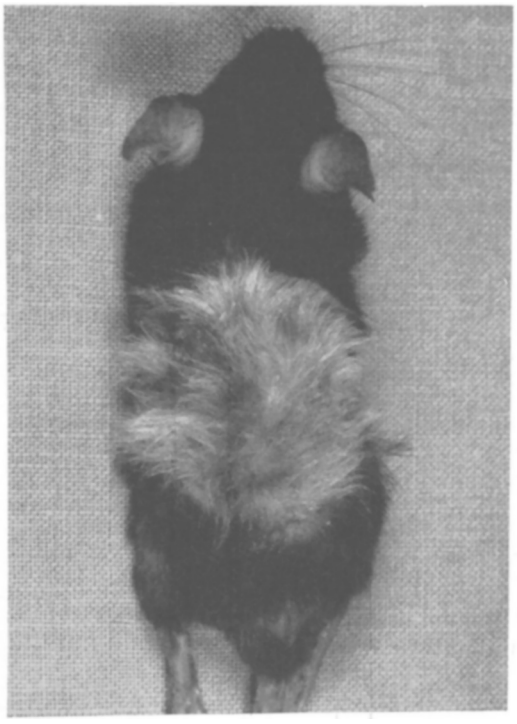

FIG. 1. Female mouse of C57Bl (Subline 1) strain bearing a large male skin isograft. Picture taken 5 mo after transplantation.

As expected, small grafts taken from (A × Z)F_1 hybrid donors and transplanted to mice of the Z parental strain failed to survive in all instances. Furthermore, grafting of large grafts in this donor-host strain combination caused death of the host, from 3 to 5 days after operation.

Transfer of tolerance induced by a large skin isograft. Recent experiments from this laboratory have demonstrated that immunological tolerance of male skin isografts induced in female mice of the C57Bl (Subline 1) strain by male antigen exposure at birth could be transferred into adult isologous females by intravenous injection of spleen cells taken from tolerant donors (unpublished). Since large male skin isografts are readily accepted by non-pretreated adult females of this strain thus demonstrating a state of immunological tolerance, it was considered desirable to ascertain whether or not this state of non-reactivity to the male could also be transferred to adult females by injecting these animals with spleen cells taken from donors accepting large male skin isografts. If this were the case it would indicate that the 2 phenomena, *i.e.*, tolerance induced by spleen cell injection at birth and that induced by a direct skin graft had similar underlying mechanisms.

The experiment was carried out as follows: 2 groups of adult female mice of the C57Bl (Subline 1) strain were used as recipients.

TABLE II. Transfer of Tolerance of Male Skin Isografts in Adult C57Bl (Subline 1) Female Mice Injected Intravenously with Spleen Cells Taken from Tolerant Females.

Group	Tolerance of male induced by:	No. of nucleated cells inj. (millions)	No. of inj. females accepting male skin/No. of grafted
I	Inj. of male cells at birth	10-30	8/8 (100%)
II	Large male skin isograft	20-30	0/6 (0%)

One group received an intravenous injection of approximately 20 to 30 million viable spleen cells taken from isologous female donors previously made tolerant of male skin isograft by antigen exposure at birth. Another group received approximately the same amount of cells taken from female donors accepting a large male skin isograft. At 10 to 15 days after treatment both groups of mice received full-thickness abdominal skin isografts taken from adult male donors. The size of the piece of skin grafted was 3 cm^2 (1.5 $\times$ 2.0 cm). After surgery mice were inspected daily for from 3 to 5 months.

The results are listed in Table II. All mice of group I injected with spleen cells taken from females tolerant of male tissue by newborn exposure were susceptible and accepted male skin isografts. In contrast, none of those which had been injected with spleen cells taken from female donors tolerating large male skin isografts were susceptible and these animals readily rejected the male skin isograft. Therefore, it seems clear that while tolerance induced at birth by spleen cell injection is transferable into isologous adult females by inoculating spleen cells taken from tolerant individuals, tolerance resulting from grafting a large piece of male skin is not transferable to another individual by injection of spleen cells.

Discussion. The results of these experiments confirm those previously reported by Zoticov and Budik who found a definite prolongation of skin homografts performed in rats when amount of skin grafted was greater than 25 cm^2. In our experiments this was found to be the case in mice as well. The prolonged survival of the foreign skin graft was, however, evident only in certain donor-host strain combinations such as in female mice of the C57Bl (Subline 1) strain grafted with large male skin isografts. In this regard, while relatively small pieces of skin taken from male donors (3 cm^2) were promptly rejected by the female recipient, those involving approximately 60% of total body surface were accepted in the great majority of cases (83%). In contrast, in other donor-host strain combinations such as Balb/C $\rightarrow$ DBA (Subline 2), DBA (Subline 2) $\rightarrow$ Balb/C or (A $\times$ Z)F_1 $\rightarrow$ Z (C3H) both small and large skin homografts were rejected in all instances.

It is interesting to note that in the combination of (A $\times$ Z)F_1 hybrid donor and Z(C3H) hosts, grafting of the large piece of homologous F_1 hybrid skin resulted in early death of the Z strain recipient. The Russian workers have also mentioned early death in a high proportion of their rats receiving large skin homografts. As in the case of the rats the early mortality observed in our group of Z strain mice receiving (A $\times$ Z)F_1 hybrid skin grafts cannot be explained on the basis of trauma and surgical manipulation since large skin isografts performed in a small group of Z strain mice resulted in acceptance of the graft in all instances without either early or delayed mortality of the host. Furthermore, no mortality due to grafting was observed in either Balb/C strain mice receiving grafts taken from DBA (Subline 2) donors, in DBA (Subline 2) transplanted with skin taken from Balb/C homologous donors or in C57Bl (Subline 1) females receiving large skin isografts taken from male donors.

The early mortality observed in Z strain animals receiving large (A $\times$ Z) F_1 hybrid skin homograft and the absence of ill effects in the other donor-host strain combinations used is difficult of interpretation. However, one might tentatively postulate that this phenomenon is related to degree of antigenic disparity between donor and host, which was slight in case of male and female mice of the C57Bl (Subline 1) or Balb/C and DBA (Subline 2) strain and stronger between (A

$\times$ Z) F_1 and Z strain mice.

Recently Trentin(7) has attributed the early mortality obtained in sublethally irradiated mice receiving homologous bone marrow transplants to a strong rejection reaction on the part of the host against the grafted cells. In our experiments a similar mechanism might account for the early death observed in Z mice grafted with large (A $\times$ Z) F_1 homologous graft. The weakness of the antigenic disparity between male and female mice of the C57Bl (Subline 1) strain and between DBA (Subline 2) and BALB/C strain mice might preclude a rejection reaction strong enough to result in death of the host. Thus, it is postulated that the death of Z mice receiving very large (A $\times$ Z)F_1 skin homograft is due to the massive rejection reaction itself.

The finding that large male skin isografts (24.5 cm^2) are accepted by females of the C57Bl (Subline 1) stock is of particular interest. In all respects the inability of the female to reject such an amount of male skin cannot be distinguished from the situation which pertains in classical immunological tolerance induced by administration of male spleen cells at birth or during adult life. In both instances the male graft is established permanently. However, the underlying mechanisms of both types of tolerance might be entirely different. In this regard, Zoticov and Budik have postulated that acceptance of large skin homografts in rats might be the result of the effect of a massive dose of foreign antigen probably contained in the grafts which leads to suppression of the immunological capacity of the host. Tolerance induced by administration of male spleen cells at birth might also result from the presence of amount of antigen which overwhelms the immunological capacity of the host, with the requirement for antigen being considerably smaller under circumstances in which the animals' total complement of immunologically competent cells is low (as in the neonatal period or following irradiation). Following spleen cell injection it is presumed that the tolerant state is maintained by release of foreign antigen by the foreign cells in residence in the recipient tolerant host. This possibility is supported by the observation that classical tolerance to a certain strain induced with spleen cells from that strain can be transferred to a new host by injection of spleen cells from the recipient tolerant animal(8). Thus the site of replication and release of the antigenic components is assumed to reside in the chimeric spleen and possibly other reticulo-endothelial tissues. It is consequently of great interest that the tolerant state induced with massive-sized skin homografts cannot be transferred by injection of spleen cells. It is presumed in this instance that the tolerant state may be induced by massive antigenic exposure from the huge skin graft and that the tolerant state is maintained by constant delivery of antigen from the skin graft itself.

It seems clear, therefore, that tolerance of male skin isograft in adult female mice can be induced by grafting the females with a male skin graft involving approximately 60% of total body surface. The antigenic disparity between host and donor as well as total amount of foreign antigen to which the host is exposed, which actually may be a function of the former, appear to be of importance in suppressing the immunological capacity of the host to react against the specific foreign antigens.

Summary. 1. Female mice of the C57Bl (Subline 1) strain capable of rejection of small male skin isografts regularly accepted large male skin isografts. 2. Large homologous skin grafts between genetically closely related strains were regularly rejected as were small skin homografts. 3. Large skin homografts between strains of mice characterized by strong histocompatibility differences regularly resulted in early death of the recipient. 4. Tolerance of C57Bl (Subline 1) female mice to male skin isografts induced by spleen cell injection could be transferred by injection of spleen cells from tolerant donors to new females during the post-neonatal period. Attempts to transfer tolerance induced with a large skin graft were unsuccessful.

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Male Skin Isograft Survival in Pregnant and Multiparous Female Mice.* (26375)

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Breyere and Barrett have demonstrated that multiparous female mice of the BALB/cAn strain which had borne litters of strain DBA/2 males became tolerant of a transplanted sarcoma originated in DBA/2 strain mice(1). Recently Prehn(2) demonstrated that female mice of the C57Bl/An strain parous by repeated matings to littermate males accepted male skin isografts in high proportion of cases. Furthermore, the incidence of tolerance of male graft was proportional to the number of litters borne by the female recipient. Thus, while 60% of the females having 1-3 litters were tolerant of male skin isografts, 90% of those having 4-6 litters were also tolerant and accepted male skin isografts.

In the experiments reported herein studies were undertaken to determine male skin isograft survival in multiparous female mice of the C57Bl (Subline 1)§ strain. Female mice of this strain regularly reject skin grafts taken from isologous males although the antigenic disparity between donor and recipient is rather weak allowing induction of tolerance in the females to male skin graft by exposure to the male antigen during the neonatal period as well as during adult life(3,4).

The experiment was carried out as follows: Female breeder mice in the C57Bl (Subline 1) inbred colony were separated from the breeder boxes after they had borne 2-6 litters (average 3.5). One group of 16 animals received a full-thickness skin graft (1.5 × 2.0 cm in size) taken from isologous males. Grafting was performed at midpregnancy (12-14 days after fertile mating). Another group of 11 mice received the male skin graft 9-16 days after delivery of last litter. A third group of 9 mice received the male skin graft 43-105 days after delivery of last litter. Finally, a fourth group consisted of 19 virgin controls receiving male skin isografts.

The technic of skin grafting as well as the criteria used to judge the success or failure of the graft were the same as previously described(5). Mice were singly housed in plastic boxes and fed Laboratory chow and tap water.

The results are listed in the Table. There was only a slight increase in male graft acceptance in the females receiving the transplant either during pregnancy or 9-16 days after delivery as compared to the virgin control group. However this difference is not statistically significant. In addition, no significant differences in rejection time of the

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§ Mice of this strain were separated from Dr. J. J. Bittner's mouse colony in 1956 and maintained in our laboratory by rigorous inbreeding.