Skin Homograft Survival in Thymectomized Mice.* (27149)

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Recently there has been increased impetus to study the role of the thymus in immunological processes. The thymus has presented an enigma, being an organ rich in lymphocytes but incapable of feeble antibody capacity(1,2,3,4,5). The thymus is maximal in relative size at an early age when immunological capacity is minimal(6). Embryologically this organ unlike other lymphoid organs develops from an endodermal sacculation of the third ventral pharyngeal pouch.

Our interest in the thymus stemmed from 2 sets of observations. The first is the high incidence of thymoma in patients with agammaglobulinemia. Seven patients have been observed who have had both thymoma and agammaglobulinemia(7,8,9,10,11). This is an association far too frequent to be explained by chance alone. Although removal of the thymic tumors does not relieve the agammaglobulinemia or the associate state of immunological incompetence, the association suggests an important link between normal thymic function and development or maintenance of immunological integrity.

The second observation is the important investigations of Mueller *et al.*(12,13) indicating that removal of or hormonal interference with the development of the Bursa of Fabricius (a lymphoid organ derived from the posterior gut in the immature chicken) interferes with the development of ultimate immunological potential.

Experiments employing thymectomy in young(14) and mature animals(9) failed to reveal evidence of participation of the thymus in the immune response in rabbits, but suggestive evidence for participation of the thymus in immunological responsiveness in immature guinea pigs was published by Fichtelius *et al.*(15). Recently Archer and Pierce (16) presented inconsistent but highly suggestive evidence of participation of the thymus in development of immunological competence in experiments in which thymectomy was carried out in neonatal rabbits. The experiments to be reported here provide support for the view that thymectomy in the neonatal mouse interferes with the development of immunological competence permitting homotransplantation of skin in certain strains of mice at an age where rejection of skin homografts is the rule in the intact and sham operated animals.

Method. Mice of the Z(C_3H), DBA/2 and Ce strains and F_1 hybrids resulting from the cross between A and Z(C_3H) and between Balb/C and DBA/2 strain mice were used.

In one set of experiments 2 groups of DBA/2 strain mice were employed. In one, thymectomy was performed at birth (0-24 hr after delivery) and in the other, at 30 days of age. The technic used to remove the thymus either in newborn or in weanling mice was the same as that described by Gross(17) which consists essentially of application of gentle suction on both thymic lobes through a longitudinal thoracic opening performed in the midline and traversing the manubrium sterni. Groups of sham operated mice were also prepared in both age groups. Mice operated at birth were raised by their own mothers and weaned at 30 days of age. At 35 days of age all mice of the different groups received a full-thickness abdominal skin homograft taken from (C × DBA/2)F_1 hybrid donors of approximately the same age. In all instances donors and recipients were of the same sex. The technic of skin grafting was the same as previously described(18). After grafting, mice were single housed in clear plastic cages and fed Laboratory Chow and tap water. Macroscopic inspection of the grafts was done 3 times a week for a pe-

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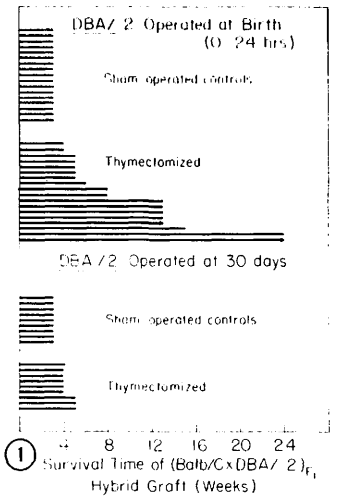
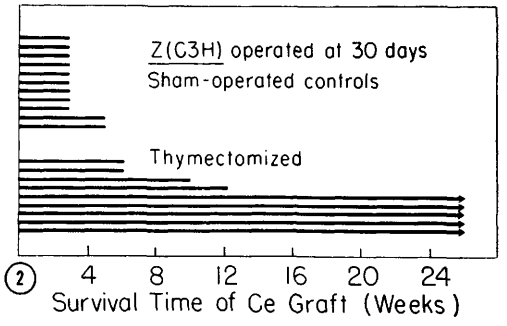
riod of at least 6 and ½ months in the longest survivors.





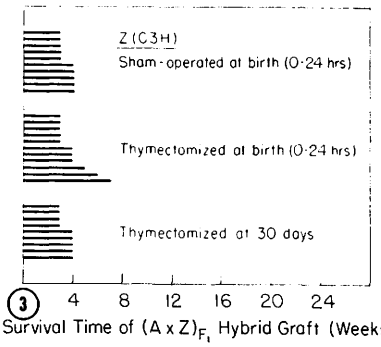


FIG. 1. Survival time of (Balb/C × DBA/2)F_1 hybrid skin homografts in DBA/2 strain mice thymectomized at birth and at 30 days of age. Each bar represents one animal. Arrows indicate grafts still surviving when report was written.

FIG. 2. Survival time of Ce skin homograft in $Z(C_3H)$ mice thymectomized at 30 days of age. Each bar represents one animal. Arrows indicate grafts still surviving when report was written.

FIG. 3. Survival time of (A × Z)F_1 hybrid skin homografts in $Z(C_3H)$ mice thymectomized at birth and at 30 days of age. Each bar represents one animal.

In another set of similar experiments $Z(C_3H)$ mice were surgically thymectomized and sham-thymectomized at birth and at 30 days of age. Mice of the latter group were grafted with homologous skin derived from either Ce or (A × Z)F_1 hybrid donors. Animals operated at birth received only (A × Z)F_1 hybrid skin homografts.

Postoperative mortality in mice thymectomized at birth ranged from 40% in those of the DBA/2 strain to 80% in those of the $Z(C_3H)$ strain. The surviving mice, however, were healthy and revealed steady growth.

Results. The results of the experiments in which DBA/2 strain mice were grafted with homologous skin taken from (C × DBA/2) F_1 donors are summarized in Fig. 1. Greatest prolongation of skin homograft survival was observed in the group thymectomized at birth. Mean survival time of the graft in this group was 10.1 weeks as compared to 3 weeks in the sham-thymectomized control group. Only a slight prolongation in homograft survival was observed in the group of mice thymectomized at 30 days of age (mean homograft survival 4.3 weeks).

The results of the experiment in which $Z(C_3H)$ mice were thymectomized at 30 days of age and grafted with homologous skin taken from Ce donors are shown in Fig. 2. The thymectomized animals maintained their grafts much longer than the sham-operated controls, mean homograft survival being 19 weeks in the former and 3.3 weeks in the latter. Five of 9 thymectomized mice still retained their Ce skin homografts in excellent condition at the time of this report (26 weeks post-surgery).

Finally, in Fig. 3 are summarized the results obtained when $Z(C_3H)$ animals were thymectomized at birth. Sham operated at birth as well as those thymectomized at 30 days of age were grafted with homologous skin taken from (A × Z)F_1 hybrid donors. Mean survival time of the skin homograft was slightly longer in those thymectomized at birth (4.1 weeks) than in either the sham-operated controls or in those thymectomized at 30 days of age. In the latter group the grafts were rejected after a mean survival time of 3.6

weeks. However, few animals in the group thymectomized at birth retained the (A × Z)F_1 skin graft significantly longer than any one animal of either the sham-operated group or those thymectomized at 30 days of age (Fig. 3).

For an understanding of the greatly prolonged homograft survival in mice of the DBA/2 strain thymectomized at birth, a preliminary experiment was performed to ascertain whether there would be changes in total and differential white blood cell counts as a result of thymectomy. Counts were performed on mice 13 weeks following thymectomy at birth and on sham-operated controls. Six mice in each group were used. The results showed that the thymectomized group had a mean WBC count of 11.000 ± 1.650/mm^3 as compared to 19.500 ± 2.500/mm^3 found in the sham-operated controls. Furthermore, differential cell counts demonstrated 6,400/mm^3 lymphocytes and 3,300 neutrophils in the thymectomized mice as compared to 15,000 lymphocytes and 3,300 neutrophils/mm^3 in the control group. Basophils and eosinophils were less than 2% of the total white cells in both groups.

Discussion. These results demonstrate that thymectomy in mice either at birth or at 30 days of age may affect the skin homograft reaction at least in certain donor-host strain combinations. For example, DBA/2 strain mice thymectomized at birth and grafted with (C × DBA/2)F_1 hybrid homologous skin showed a significant prolongation of mean skin graft survival time as compared to that in a group of control mice in which thymectomy was only simulated. On the other hand, when DBA/2 strain mice were thymectomized at 30 days of age, only a slight prolongation of mean survival time was observed. Thymectomy performed in weanling Z(C_3H) mice which were subsequently grafted with homologous skin taken from Ce donors resulted in marked prolongation of mean survival time of the graft as compared to that of the sham-thymectomized group (19.0 and 3.3 weeks, respectively). Of the thymectomized mice of this group 56% of the animals retained their grafts longer than 26 weeks and for all practical purposes could be considered as tolerant of the Ce homologous skin graft.

The results with Z(C_3H) mice thymectomized either at birth or at 30 days of age and grafted with (A × Z)F_1 hybrid homologous skin indicate that in this donor-host strain combination thymectomy performed at birth resulted in a slight but still insignificant prolongation of mean survival time of the (A × Z)F_1 skin homograft. However, a few of the animals of this group retained the homologous F_1 hybrid graft for an unusually long time suggesting that even when a strong histocompatibility barrier exists thymectomy at birth may have some influence on graft rejection.

It is interesting that DBA/2 and Balb/C as well as Z(C_3H) and Ce strain mice are isogenic with respect to the H-2 histocompatibility locus whereas those of the Z(C_3H) and A strains differ at this locus. This might perhaps explain why thymectomy was more effective in DBA/2 grafted with (C × DBA/2)F_1 and in Z(C_3H) grafted with Ce skin than it was when thymectomized Z(C_3H) mice were grafted with (A × Z)F_1 hybrid skin. In other words, it appears that early thymectomy serves to depress immunological capacity sufficiently to overcome a weak histocompatibility difference but not enough to bridge the strong difference caused by different H-2 histocompatibility genes.

It is also of interest that thymectomy in DBA/2 mice receiving (C × DBA/2)F_1 skin homografts was most effective when performed at 30 days of age. This agrees with the findings of Archer and Pierce demonstrating a deficient immunological response to bovine serum albumin in adult rabbits only when these animals were deprived of their thymus during the neonatal period.

Even with the present observations understanding of the role of the thymus in the skin homograft reaction in mice requires extensive experiments analyzing the effect on the cellular processes and immunological events involved in the homograft reaction. The very preliminary data presented here on the effect of thymectomy at birth on peripheral leukocyte and lymphocyte count recall similar observations(19,20), and should be further in-

vestigated. Whether thymectomy at birth achieves this altered immunological state through humoral or cellular mechanisms requires further study.

Whatever the final explanation, it seems clear that at least in certain donor-host strain combinations of mice isogenic with regard to the H-2 histocompatibility locus but homologous with respect to other histocompatibility genes, thymectomy of the host performed either at birth or at an early age produces a marked prolongation of the skin homograft survival.

Summary. The effect of thymectomy performed in mice either at birth or at 30 days of age on survival time of homologous skin grafts was studied. The results demonstrate that in certain donor-host strain combinations isogenic at the H-2 locus such as DBA/2 with (Balb/C × DBA/2)F_1 and Z(C_3H) with Ce, previous thymectomy of the host resulted in prolonged survival of the skin homografts. However, in other strain combinations differing at the H-2 locus such as Z(C_3H) with (A × Z)F_1 hybrid, thymectomy of the recipient resulted in a slight and perhaps unsignificant prolongation of skin homograft survival.

While this manuscript was in press similar observations were reported by Miller(21).

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Independence of Water Content and Respiration in Rabbit Erythrocytes.* (27150)

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Previous studies on the effects of changing extracellular osmolarity on erythrocyte respiration have shown varying results depending on the animal investigated, degree of extracellular hypo- and hyperosmolarity, and control of substrates. Hunter(1) demonstrated irreversible depression of respiration of chicken erythrocytes in markedly hypertonic solutions and little or no effect in hypotonic solutions. Tipton(2) found no significant change in respiratory activity of chicken

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