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Received June 11, 1962. P.S.E.B.M., 1962, v111.

Further Studies of Suppression of the Homograft Reaction by Thymectomy in the Mouse.* (27729)

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Surgical ablation of the thymus is a valuable approach to demonstration of the role of this gland in the immune response in several species of mammals. The immunologic-depressive effect of thymectomy appears to be a function of the age at which the thymus is removed and of the difference in immunological maturation at birth in the species studied (1-4). In experiments to ascertain the inhibiting effect of neonatal thymectomy in development of the capacity to reject skin homografts it was initially observed(5,6) that prolongation of skin graft survival was produced only in strains of mice sharing the H-2 histocompatibility locus and homologous with regard to other genetic loci controlling "weaker" factors. Subsequently we established that complete thymectomy performed in mice immediately after birth allows acceptance and growth of tumor tissue originally developed in a mouse differing at the H-2 histocompatibility locus(7,8). These results together with the virtually complete inhibition of immunological response to the bacteriophage T_2 in mice completely thymectomized at birth and the demonstration that small remnants of thymus tissue permitted an appreciable antibody synthesis(4) prompted us to repeat the skin homotransplantation studies in thymectomized mice and to evaluate their immunological response when confronted with normal homologous tissues possessing a pronounced difference in antigenic constitution. In the present report we demonstrate that complete thymectomy in neonatal mice produces sufficient inhibition of immunological maturation to permit acceptance of skin homografts even when the antigenic disparity between the strains of animals studied is conditioned by the H-2 histocompatibility locus. These experiments also demonstrate that when thymectomy is carried out in weanling mice no effect on skin homograft survival is demonstrable if the strains of mice differ at the H-2 histocompatibility locus whereas prolonged survival of skin homografts is sometimes observed when the homologous mice share the antigens controlled by H-2 locus. Finally it is shown that complete thymectomy at birth permits female mice of the C57Bl strain to accept at a later date skin isografts from male donors, thus establishing that neonatal thymectomy permits them to overcome the Eichwald-Silmser(9) histocompatibility barrier.

Material and methods. Inbred mice of the C57Bl/1, DBA/2 and C3H/Bi strains and F1 hybrids resulting from the cross between Balb/C and DBA/2 and between A and C3H strains were employed. Groups of mice of the C57Bl/1, DBA/2 and C3H strains were thymectomized or sham-operated either at birth or 35 days later. At this later age, groups were also prepared in which both thymus and spleen were removed. The technic for thymectomy in newborn mice was essentially the same as that described by Dischler and Rudali(10) and the procedure employed in older animals was the same as that previ-

* Aided by grants from U.S.P.H.S., Am. Cancer Soc. and National Foundation.

TABLE I. Effect of Thymectomy on Capacity of Mice to Reject Skin Homografts.

		Type of operation in host and age at which it was performed				
Host strain	Skin donor strain	Thymectomy at birth	Sham-thymect. at birth	Subtotal thymect. at birth	Thymect. at 35 days	Thymect. + splenect. at 35 days
C57Bl/1 ♀	C57Bl/1 ♂	6/6*	1/15*		1/12*	0/7*
DBA/2	(Balb/C × DBA/2)F1	8/8	0/12		0/11	
C3H	(A × C3H)F1	12/14	0/23	0/10	0/14†	0/30†
DBA/2	C3H	9/12	0/19	0/9		

* No. of mice accepting skin/No. grafted.
† Skin was grafted 1 wk or 2 mo after surgery.

ously used by Gross(11). Ether anesthesia was employed and the thymic lobes were removed applying suction with a glass aspirating pipette to each lobe. Beginning at time of operation mice were weighed at weekly intervals on a Mettler balance until the end of the experiment. Animals operated at birth were raised by their own mothers, weaned at 30 days of age and skin grafted 10 days later. Mice operated at 35 days were generally grafted 1 week later and in 2 groups 2 months later. Full-thickness abdominal skin was obtained from 2- to 3-month-old donors. The technic of grafting was the same generally used in this laboratory and previously described(12). Groups of operated recipients and the corresponding donors are shown in Table I. Among these combinations, DBA/2 with C3H and C3H with (A × C3H) F1 differ from one another at the H-2 histocompatibility barrier since DBA/2 are H-2^d, C3H are H-2^k and A strain mice are H-2^a. With the exception of the experiment involving C57Bl/1 mice, donors and hosts were always of the same sex. Mice were observed for general appearance and status of the skin graft at least once every other day.

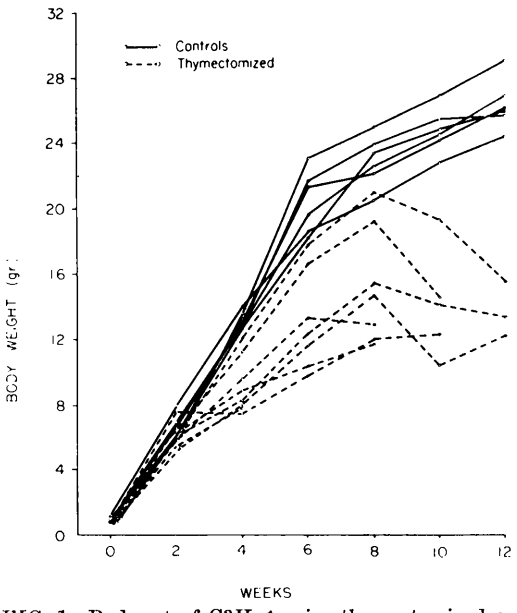

FIG. 1. Body wt of C3H ♂ mice thymectomized or sham-operated within 24 hr of birth.

Results. Fig. 1 shows the body weight changes of a group of C3H mice either thymectomized or sham-operated during the first day of life. It is apparent that after the second week of age weight gain of thymectomized animals was abnormally low. This effect has also been observed in other strains of mice studied when the thymus is completely extirpated immediately after birth. In contradistinction, thymectomy in weanling mice had no significant effect on growth rate.

Twenty-one C3H and 18 DBA/2 strain mice which had been completely thymectomized immediately after birth were weaned at 30 days of age. Most of them had been seriously ill with diarrhea during the second and third weeks of life, which only occasionally was noted among normal or sham-operated mice. Further, these animals regularly developed hunched appearance, roughing of the fur and diarrhea during the second or third month of life and finally died, apparently of intercurrent infections, generally before the fourth month of life. Mean death time (± S.D.) was 56 ± 18 days in the C3H strain and 67 ± 22 days in the DBA/2 strain. A smaller group of C57Bl/1 mice thymectomized at birth also revealed apparent inability to resist infection and early death. Mortality in non-operated mice as well as in animals that were subtotally thymectomized

or sham-operated at birth, or thymectomized at 35 days of age was always less than 5%.

Table I summarizes the results of experiments in which operated animals were grafted with homologous skin. The short survival of mice thymectomized immediately after birth did not always permit observation of their skin homografts for a sufficiently long time to permit evaluation of the success or failure of the transplants. To overcome this difficulty results have been tabulated on only those animals surviving longer than 35 days after grafting. Animals dying sooner, either accepted the skin homografts or failed to develop vascularization of the grafted tissue, the latter being a primary failure, due in all probability to the poor general condition of the animals and not to homotransplantation immunity. In the first experiment, females of the C57Bl strain which were either sham-operated at birth or subjected to thymectomy or to thymectomy and splenectomy at 35 days of age rejected in most cases the isologous male skin, whereas 100% of the animals subjected to thymectomy before 24 hours of age accepted this skin.

In the second experiment DBA/2 mice accepted the homologous (Balb/C × DBA/2)-F1 skin only if they were subjected to thymectomy in the neonatal period. In these experiments mice thymectomized 35 days after birth sometimes exhibited a slow homograft rejection. As a group, however, the rejection time of homotransplants in the groups thymectomized as weanlings did not differ significantly from that of controls. Nonetheless, it is of interest that 2 DBA/2 mice thymectomized at 35 days of age kept (Balb/C × DBA/2)F1 skin homografts on for more than 8 weeks, a result never observed among many homografts carried out between non-thymectomized members of these 2 strains.

In the experiments with C3H mice homotransplanted with (A × C3H)F1 skin the results are clear cut. It will be seen from the table that 86% of the mice in which the thymus was completely removed on the first day of life accepted the homografts whereas sham-thymectomy or incomplete thymectomy (removal of 90 + % of the thymus) at birth did not interfere with the homograft rejection. When this strain combination was studied neither thymectomy nor thymectomy + splenectomy carried out in weanling mice (35 days of age) altered the capacity of these animals to reject skin homografts.

Finally 75% of the DBA/2 mice thymectomized at birth when transplanted at 40 days of age accepted the homologous skin taken from C3H donors. By contrast mice subjected to sham-thymectomy or partial thymectomy at birth always rejected the skin homografts.

In both these experiments when either the C3H or the DBA/2 animals, completely thymectomized at birth, did reject the skin homografts, the rejection was abnormal, beginning late, and presented a more protracted rejection than that observed among the sham-operated controls.

Discussion. These results confirm previous observations(5,10,13,14) indicating that removal of the thymus in neonatal mice impairs their capacity to resist common infections and to grow steadily. In fact all the animals which were deprived of their thymus within 24 hr after birth ultimately developed disease and died before the fourth month of age. Secondly, they demonstrate that the Eichwald-Silmser sex linked histocompatibility barrier to isotransplantation can be overcome by thymectomy in the neonatal period. Further, these results indicate that complete thymectomy in the neonatal mouse permits homotransplantation of skin not only across relatively weak histocompatibility barriers as those between DBA/2-(Balb/C × DBA/2)-F1 host donor combination but also across the strong histocompatibility barriers conditioned by the H-2 histocompatibility locus such as those existing between DBA/2 and C3H and between C3H and (A × C3H)F1 hybrids. Thymectomy in weanling mice had a very feeble effect on homotransplantation immunity but did occasionally appear to alter reactivity sufficiently to permit prolonged survival of skin homografts when the grafting was carried out between strains of animals that differ from one another by weak histocompatibility barriers.

These results are in agreement with previous experiments from this laboratory showing

that mice thymectomized in early life are capable of accepting homografts of malignant tissue spontaneously arising from a mouse differing from the recipient host at the H-2 histocompatibility locus. The contradiction of our present data with those previously reported(5,6) suggesting that in mice the skin homograft rejection is only slightly influenced by neonatal thymectomy in those combinations differing at the H-2 histocompatibility locus is best explained by improvements in thymectomy technic. This interpretation is supported by observations reported herein indicating that when even as little as 5-10% of the thymus is left in place, homotransplantation immunity proceeds in almost normal fashion. Further, these observations agree well with other studies from our laboratory which have indicated that complete thymectomy at birth in mice virtually eliminates capacity to form antibodies against the T_2 choli bacteriophage whereas significant amounts of antibody are produced when even small remnants of the thymus have been left in place (4).

The results herein reported are in keeping with experiments from this and other laboratories indicating that spleen cells derived from adult mice thymectomized shortly after birth fail to elicit graft *vs.* host reactions(15) and that animals deprived of their thymus early in life are more susceptible to runting disease when injected with homologous immunologically competent cells(13,14) and fail to develop hypersensitivity reactions of the delayed type(16). It seems safe to conclude that in mammals the thymus gland plays a key role in development of full immunological capacity. The modus operandi of this key role is under intensive investigation.

Summary. 1. Mice thymectomozid immediately after birth do not grow normally, fail to resist common infections, and die before the 4th month of life. 2. Thymectomy performed within 24 hours after birth allows the acceptance of skin homografts in all donor-host combinations studied, even in those differing at the H-2 histocompatibility locus, such as the C3H with (A × C3H)F1 and the DBA/2 with C3H. 3. Thymectomy at 35 days of age results in slight prolongation of skin homograft survival only in few animals in strain combinations sharing the H-2 locus.

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Received June 11, 1962. P.S.E.B.M., 1962, v111.