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Failure of Spleen Cells from Thymectomized Mice to Induce Graft *vs.* Host Reactions.* (27466)

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The role of the thymus in development of immunological capability has been the subject of a series of significant investigations in recent years. It was initially observed that abnormality of the thymus gland (benign thymoma) is associated with agammaglobulinemia(1) in a frequency far too high to be explained by chance association(2,3). Fichtelius *et al.*(4) in guinea pigs and Archer *et al.*(5,6) in rabbits presented evidence indicating that thymectomy performed in early life interfered with development of immunological potential. Subsequently, Miller(7) and Martinez *et al.*(8) showed that thymectomy in the neonatal period in mice interferes with the development of the capacity of mice to reject skin or tumor(9) homografts. Glick *et al.*(10) and Mueller *et al.*(11,12), established that removal of the bursa of Fabricius in newly hatched chickens or hormonal suppression of the development of this organ inhibited full development of immunological potential of these animals. Papermaster *et al.* (13-15) also showed that hormonal suppression of the development of the bursa of Fabricius interfered with full development of the homograft reaction in young chickens permitting more vigorous immunological activity and graft *vs* host reaction following transplantation of adult chicken spleen cells stimulated with antigen *in vitro*.

In addition to these findings, observations of thymectomized mice in this laboratory have revealed that animals completely thymectomized shortly after birth failed to grow normally and regularly developed disease and wasting during the second month of life. These animals often died apparently of intercurrent infections. Studies employing the

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T_2 bacteriophage as an antigen indicated that mice thymectomized in the neonatal period are much less capable than normal mice of forming neutralizing antibody against this antigen(16).

To study further the immunological capacity of lymphoid cells derived from mice submitted to thymectomy in early life the method described by Simonsen(17,18) as the graft *vs* host assay of histocompatibility was employed. In essence these experiments show that peripheral lymphoid cells obtained from the spleen of animals which have been completely thymectomized as neonates are incapable of producing graft *vs* host reactions in F1 hybrid recipients.

Materials and methods. Mice of the A and Z‖ strains and F1 hybrids resulting from the cross between A and C57Bl, Z and C57Bl and between Z and DBA/2 strains were used. Mice of the A and Z strains were either thymectomized or sham operated during their first day of life or at 6 days of age. Two months after the operation female mice were sacrificed and the immunological competence of their spleen cells was studied employing the Simonsen assay, which essentially consists of determining the spleen enlargement induced in suitable recipients as a result of transplantation of adult spleen cells. In this assay the recipients are young first generation hybrids resulting from the cross between the donor strain and another strain against which the cells of the donor are expected to react. Eight-day-old litters of 6 to 9 hybrid mice were employed for assay. To assure maximum uniformity among recipients each litter was divided into 3 groups to be injected with spleen cells obtained either from a) normal F1 hybrids isologous with the recipients, b) sham thymectomized parental strain mice homologous to one strain included in the hybrid cross, and c) thymectomized parental strain mice from the same strain as that employed in "b".

The donor animals were killed with ether and their spleens were made into suspensions in Ringer-Locke's saline solution containing approximately 10 million viable spleen cells per 0.1 cc. These suspensions were immediately injected intraperitoneally into the 8-day-old F1 hybrid in a dose of 0.1 cc per mouse. Eight days later they were sacrificed and their body and spleen weights recorded. The following graft *vs* host assays of adult lymphoid cells taken from animals that had been operated immediately after birth were performed: 1) A strain cells into the (A × C57Bl)F1 hybrid, 2) Z strain cells into the (Z × C57Bl)F1 hybrid, and 3) Z strain cells into the (Z × DBA/2)F1 hybrids. In a final experiment, spleen cells from 2-month-old Z mice that were thymectomized or sham operated at 6 days of age were tested in the (Z × DBA/2)F1 hybrid mice. In all studies the spleen weight per 100 g of mouse and the spleen index were determined and recorded. The "spleen index" is obtained by dividing the mean weight of spleen of F1 hybrid recipient 8 days after injection of adult spleen cells from experimental animals by the mean weight of spleen of F1 hybrid recipient 8 days after injection of adult spleen cells from F1 hybrid controls.

Results. The results are summarized in Table I. The spleens of the (A × C57Bl)F1 hybrid mice which received cells from A strain mice submitted only to sham operation during their first day of life weighed on the average more than twice as much as did those of their littermates injected with isologous F1 hybrid cells (index: 2.27) whereas the spleens of other littermates injected with cells from A strain mice thymectomized immediately after birth presented approximately the same weight as those of the control animals (index: 0.98). Entirely comparable results were obtained when cells from sham operated and thymectomized Z mice were tested in the (Z × C57Bl)F1 and in the (Z × DBA/2)F1 hybrids. The results were the same regardless of whether the Z mice had been thymectomized at 1-24 hours or at 6 days after birth. The t-test was applied to these results and for every group statistical analysis demonstrates that the differences in spleen weight of hybrid mice receiving cells from thymectomized animals and those injected with cells originating from isologous F1 hybrid mice

‖ Z is used to designate mice of the C_3H strain originally maintained by Dr. John J. Bittner.

TABLE I. Graft *vs* Host Assay of Spleen Cells from Thymectomized and Sham Operated Mice in First Generation Hybrids.

Host strain*	Spleen donor strain	No. of mice inj.	Spleen wt, mg/100 g body wt	Spleen index (exp./control)	Body wt index (exp./control)
(A × C57Bl)F1	(A × C57Bl)F1	19	632 ± 31†	—	—
	A Sham operated	17	1435 ± 92	2.27	.93
	A Thymect. (1-24 hr)	16	621 ± 42	.98	.97
(Z × C57Bl)F1	(Z × C57Bl)F1	11	426 ± 33	—	—
	Z Sham operated	7	1074 ±120	2.52	.97
	Z Thymect. (1-24 hr)	6	449 ± 67	1.05	.95
(Z × DBA/2)F1	(Z × DBA/2)F1	9	433 ± 38	—	—
	Z Sham operated	11	933 ± 60	2.30	1.05
	Z Thymect. (1-24 hr)	5	460 ± 63	1.06	1.00
	Z " (6 days)	8	475 ± 14	1.10	1.14

* Each mouse was inj. intraper. at 8 days of age with 10 million donor spleen cells and killed 8 days later.
† Mean ± S.E.

are not significantly different. By contrast, the spleen weights of animals receiving cells from sham operated mice were always significantly greater than those of the control animals.

Discussion. The results of these experiments demonstrate that spleen cells from adult parental strain mice thymectomized early in life are incapable of producing spleen enlargement in young F1 hybrids resulting from the cross between mice isologous with the donor strain and another line against which the sham operated donors showed a consistent and vigorous homograft reaction. This lack of immunological competence, as revealed by an inability to elicit conspicuous manifestations typical of the graft versus host reaction, was found to be characteristic not only of adult mice thymectomized during their first day of life, but also in Z strain animals when thymectomy was performed at 6 days of age.

These observations are in line with previous experiments demonstrating that thymectomy performed in neonatal mice leads to an immunological depression of sufficient degree to allow the survival of tissue homografts, even in those donor-host combinations with the strong histoincompatibility conditioned by a difference at the H-2 locus, using both neoplastic(9) or normal tissues (Dalmasso *et al.*, unpublished).

It is important to realize from the experiments reported herein that transfer of lymphoid cells from the spleens of thymectomized animals even to an environment of an intact non-thymectomized host does not restore the immunological integrity of these cells. Instead these experiments further reflect the immunological inadequacy of the spleen cells of animals thymectomized at birth. This observation may have important bearing on hypothesis concerning the nature of the essential role played by the thymus in immunological development. The observations fit well with the hypothesis previously expressed from this laboratory that the thymus in mammals and bursa of Fabricius in chickens may play a critical role in centrifugal distribution to the other lymphoid organs and lymphoreticular tissue of cells possessing potential immunological competence.

It appears clear that in mice, rabbits and guinea pigs the lymphoid tissue of the thymus and of the bursa of Fabricius in chickens plays in early life a prominent role in attainment of full immunological capacity. The mechanism by which this function is accomplished has not been established. The thymus as stated above might be a source of origin of immunologically competent cells during late gestational and early neonatal life or it might produce hormonal factor(s) which stimulate immunological maturation of the lympho-reticular system in the early stage of its development. Although we would consider the results of these investigations to favor the former hypothesis they would not be

incompatible with either mechanism of operation of this organ.

Summary. 1. The capacity of spleen cells from mice thymectomized during neonatal life to elicit graft *vs* host reactions was studied using Simonsen's graft *vs* host assay of histocompatibility. 2. The reaction of A strain spleen cells from mice sham operated or thymectomized in the neonatal period was compared to that of isologous F1 cells in (A × C57Bl)F1 recipients and Z strain spleen cells from similarly treated groups were compared in (Z × C57Bl)F1 and in the (Z × DBA/2)F1 hybrids. 3. The results show that early complete thymectomy in both A and Z mice renders the spleen cells of these animals incapable of producing graft *vs* host reactions in F1 hybrids. 4. These data are interpreted as evidence that the cells themselves of the lympho-reticular system in mice thymectomized at or shortly after birth are immunologically defective. 5. These observations are considered as a further evidence that the thymus plays a key role in development of immunological capacity in mammals.

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Inhibition of P^{32} Incorporation into Rabbit DNA *in vivo* by Deuterium Oxide.* (27467)

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Animals which have been given deuterium drinking water show widespread effects: impairment of kidney function, anemia, disturbed carbohydrate metabolism, central nervous system disturbances, altered adrenal function and, when approximately one-third of their body water has been replaced by D_2O, death(1). Incubation of rabbit bone marrow cells *in vitro* with deuterium oxide has been reported drastically to inhibit the synthesis of DNA in these cells(2). Moreover, it has been suggested that many of the biological effects of D_2O may be due to alteration of the ordered helix-random coil equilibrium in DNA secondary structure(3).

In view of these findings, we have investigated the effects of deuterium on DNA synthesis *in vivo,* and the effects of deuteration

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