

zymatic activity. It may be that the H-2 antigens are part of the structural material of the cell membrane(13).

Summary. The distribution of H-2 specificities on LM mouse cells and derived lines was studied using an immunofluorescent technique. All specificities of the *H-2^k* allele were found to be distributed uniformly among cells of all lines studied regardless of the presence or absence of alkaline phosphatase and of marker chromosomes. Variations in modal number of chromosomes and in morphology and growth characteristics of the cells also were unrelated to the presence of H-2 antigens.

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Increased Graft-*Versus*-Host Susceptibility of Thymectomized Recipients.* (31754)

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As determined by the Simonsen assay of histocompatibility(1), spleen cells derived from adult mice, thymectomized shortly after birth, fail to elicit graft-*versus*-host reactions. Previous experiments in this(2) and other laboratories(3) indicate that early thymectomy of the recipient facilitates and increases the severity of the immunological disease produced by administration of normal splenic cells.

Gross spleen enlargement after inoculation of immunologically competent cells has pro-

vided a sensitive assay system for measurement of GVH reactions by cells capable of interacting with antigens present in the host. Earlier studies(2) in our laboratory indicate that neonatally thymectomized mice are more susceptible to the lethal effects of graft-*versus*-host reactions than are normal controls. Recent work(4,5) has shown also that host cells make a major contribution to the proliferative response of spleen and other lymphoid tissues undergoing the graft-*versus*-host responses. It seems of interest, therefore, to determine whether neonatally thymectomized mice can respond with the development of splenomegaly following injection of splenic cells from adult immunologically competent donors. Experiments were designed to compare the susceptibility of thymectomized and sham operated recipients to produce splenomegaly. In this paper we attempt to demonstrate that thymectomy of the recipient ani-

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TABLE I. Results of Graft-*versus*-Host Assay in Neonatally Thymectomized and Normal F₁ Recipients.

Group	I.P. inj of 30 million adult spleen cells	Spleen indexes*										Mean $\pm$ S.E.
(C3H $\times$ A)F ₁ neonatal thymectomy	A	2.17	2.20	2.46	2.97	3.03	3.59	4.00	4.21	4.78		3.27 $\pm$.31
	C3H	1.33	1.48	1.68	1.85	1.92	2.31	2.54	2.59	2.84		2.06 $\pm$.17
	No injection	.72	.77	.83	.92	.97	.97	1.07	1.11			.92 $\pm$.04
(C3H $\times$ A)F ₁ intact mice	A	1.12	1.43	1.51	1.58	1.70	1.73	1.80	2.00	2.01		1.84 $\pm$.12
		2.07	2.42	2.76								
	C3H	.84	1.00	1.01	1.03	1.04	1.04	1.12	1.12	1.26		1.16 $\pm$.06
		1.27	1.48	1.65								
	No injection	.75	.81	.84	.84	.92	.93					.84 $\pm$.02

* Spleen indexes were calculated by dividing relative spleen weight (mg/100 g body wt) of the experimental groups by relative spleen weight of the negative control group (mice injected with 30 million spleen cells from 2-month-old (C3H $\times$ A)F₁ hybrid mice).

mals in the neonatal period favors the development of splenomegaly following injection of immunologically competent cells.

Method. Mice of the C3H and (C3H $\times$ A)F₁ hybrid were thymectomized in the neonatal period. In sham operations, the thorax was opened, the thymus manipulated but not removed.

Two experiments were performed: a) Graft-*versus*-host assay of Simonsen(1) with F₁ hybrid recipients. In a first experiment, litters of 8, 8-day-old (C3H $\times$ A)F₁ hybrids either thymectomized or sham operated were divided into 4 groups of 2 mice each in order to assess the capacity of the recipients to exhibit spleen enlargement after injection of parent strain cells. Intraperitoneal injections of 30 million spleen cells from 2-month-old parent strain A or parent strain C3H were given to 2 mice of each litter. Negative controls consisted of groups of 2 mice from each litter injected with 30 million spleen cells from 2-month-old (C3H $\times$ A)F₁ hybrids.

b) Graft-*versus*-host assay of Simonsen with young C3H mice. In a second experiment, litters of 6 to 8, 8-day-old animals, either thymectomized or sham operated, were divided into 3 groups of 2 or 3 mice each. One group of mice from each litter was injected intraperitoneally with 30 million spleen cells from 2-month-old A mice. Negative controls consisted of groups of 2 or 3 mice injected with spleen cells obtained from 2-month-old C3H mice.

In both experimental groups of thymecto-

mized and intact animals, one or two mice were left uninjected. Eight days after cell administration, the animals were sacrificed, their body and spleen weights determined and the relative spleen weight (mg/100 g body weight) calculated. Finally, the spleen index was determined by dividing the mean relative weight of the experimental group by the mean relative spleen weight of the negative controls.

In thymectomized mice the absence of mediastinal thymic tissue was verified under the dissecting microscope and by microscopic examination of the mediastinal contents. Animals found to contain thymus remnants were excluded from the analysis.

Results. a) *Graft-*versus*-host reaction in F₁ hybrids.* Table I shows the results obtained using F₁ hybrid recipients. It can be seen that spleen cells from adult A mice can induce significant splenomegaly in both thymectomized and non-thymectomized recipients. Spleen cells from adult C3H mice, on the other hand, can induce significant splenomegaly only if the recipients are neonatally thymectomized. Comparisons between the spleen indexes obtained from neonatally thymectomized (3.27 $\pm$.31) and from non-thymectomized (1.84 $\pm$.12) F₁ hybrids injected with A spleen cells indicate greater splenomegaly in the thymectomized group ($p < .005$). Also, when comparing the spleen indexes of thymectomized (2.06 $\pm$.17) and non-thymectomized (1.16 $\pm$.06) hybrids injected with spleen cells from C3H mice there was a significant difference of $p < .005$.

b) *Graft-versus-host reaction in C3H recipients.* The graft-*versus*-host reaction of Simonsen was used in thymectomized and non-thymectomized 8-day-old C3H mice. The injection of A spleen cells into 8-day-old C3H mice does not produce splenomegaly ($1.11 \pm .04$) but when the recipients are thymectomized significant splenomegaly with a spleen index of $3.13 \pm .27$ is obtained (Table II). Statistical comparison of the mean of thymectomized C3H injected with A cells and the mean of non-thymectomized C3H injected with A cells shows significance at the level of $p < .005$.

In the 2 groups of experiments, thymectomized and non-thymectomized mice were left noninjected. The mean of the noninjected groups was always less than 1.0. When all relative spleen weights of noninjected mice were compared against the relative spleen weights of mice injected with syngeneic spleen cells (C3H or (C3H $\times$ A) F_1) it was found that the injected mice had greater relative weights of their spleen than did the noninjected mice ($p < .02$). These results indicate clearly that negative controls in our experiments and those of others in this assay should always be the mice injected with syngeneic spleen cells and not those left uninjected.

Discussion. The results demonstrate that thymectomy, performed immediately after birth in F_1 hybrid mice, increases susceptibility of the mice to develop splenomegaly after injection of immunocompetent cells from adult mice that are syngeneic with one of the parents. Furthermore, the injection of either C3H or A spleen cells into thymectomized (C3H $\times$ A) F_1 hybrids produces a greater splenomegaly than that found in control animals. On the other hand, significant splenomegaly can also be induced in thymectomized animals injected with the allogeneic spleen cells. We had previously reported(6) that attempts to reconstitute neonatally thymectomized C3H mice by intraperitoneal injection of spleen cells, by thymus cells or by intraperitoneal injection of subcutaneous grafting of spleen from A mice were not successful. The production of splenomegaly in the recipients here reported by the injection of spleen cells obtained from adult A mice is a further

proof that development of graft-*versus*-host reactions is facilitated by thymectomy and imposes a strict limit on the kinds of cells that can be used to reconstitute the neonatally thymectomized mouse. By implication, these results indicate the great susceptibility of the athymic recipient for graft-*versus*-host or homologous disease. The requirements for a potential GVH reaction are fulfilled by neonatal thymectomy: a) immunocompetent cells of the graft capable of interaction with antigen of the host, b) presence of antigens of the host foreign to the graft, and c) generalized depression of the immunologic capacity of the host so that the immunocompetent host cells do not reject the grafted cells. That there is host cell proliferation in GVH reactions was first demonstrated in chicken by Biggs and Payne(4), and in mice by Davies and Doak (5). Our present experiments demonstrate that in thymectomized animals of strains shown to be deficient in capacity to exercise graft-*versus*-host reactivity(7), the injection of allogeneic cells results in development of splenomegaly. These results suggest further, that the proliferation of host cells contributing to organomegaly in graft-*versus*-host reactions does not involve in a major way thymus dependent immunocompetent cells of the host. Results of this sort are important in working out the relationships of the grafted cell-host cell interactions, because recent clinical experience has indicated that patients with Swiss-type agammaglobulinemia readily develop fulminating wasting and runting homologous disease as a consequence of injection of parental bone marrow(8), foreign bone marrow(9,10) or even fetal liver cells(11). If the animal or patient is to be reconstituted following treatment with cells from a foreign donor the great hazard of homologous disease reflected in increased susceptibility to lethal runt disease in parent to F_1 transplantation, and from transplantation of homologous immunologically competent cells must be realized and controlled.

Summary. Thymectomy performed immediately after birth increases the susceptibility of (C3H $\times$ A) F_1 recipients to development of splenomegaly following the injection of spleen cells from A and C3H mice, and of

TABLE II. Graft-*versus*-Host Assay in C3H Neonatally Thymectomized Mice.

Group	I.P. inj of 30 million spleen cells from	Spleen indexes*	Mean spleen index $\pm$ S.E.
C3H thymecto- mized mice	A mice	2.50—2.66—2.81—3.05—3.37—4.37	3.13 $\pm$.27
	Non-injected	.75—.76—.95—.96—.98—1.06	.91 $\pm$.27
C3H intact mice	A mice	1.01—1.02—1.08—1.18—1.24	1.11 $\pm$.04
	Non-injected	.80—.91—.93—.98—1.14	.95 $\pm$.05

* Spleen indexes were calculated by dividing relative spleen weight (mg/100 g body wt) of the injected and non-injected groups by relative spleen weight of the negative control group (syngeneic spleen cell injected mice).

C3H recipients to produce splenomegaly following the injection of spleen cells from A mice. In view of current knowledge, that much of the splenomegaly in graft-*versus*-host reaction is due to proliferation of host cells, our experiments indicate that this proliferation does not involve thymus dependent immunocompetent cells of the host. These findings stress once again the susceptibility of the neonatally thymectomized recipient mouse to the development of homologous disease after injection of homologous cells which are readily rejected by the intact recipients. The findings have important implication in efforts to design and execute replacement therapy for humans genetically or developmentally lacking in thymic system of lymphocytes.

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Alimentary Absorption of L-Arabinose and D-Xylose in Chicks.* (31755)

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While most of the previous investigations on alimentary absorption of sugars pertain to the mammalian species, Bogner(1) and Bogner and Haines(2) experimented on newly hatched and very young chicks with a view to observing the rates of absorption of several sugars and suggested that the selective ab-

sorption capacity of the chick's gut is not maximally developed until one to three days after hatching.

The aims of the present investigation were: (a) to determine the absorption rate of L-arabinose and D-xylose as compared to D-glucose; (b) to study the effect of age on the relative absorption; and (c) to observe the effects of concentration on absorption rate.

Materials and methods. The chicks used were New Hampshire $\times$ Columbian males

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