STUDIES ON IMMUNOLOGIC RECONSTITUTION OF THYMECTOMIZED MICE*

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Plates 50 to 53

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Recent investigations have established that the thymus is essential for full development of immunologic responsiveness in mammals, particularly in rodents. The immunologic effects of neonatal thymic ablation have been particularly well characterized in the mouse: atrophy of the lymphoid tissues, depletion of lymphocytes in the peripheral blood, and extremely deficient transplantation immunity permitting grafting of allogeneic and even xenogeneic tissues (1, 2). Antibody production is variably affected, depending apparently on the character of the antigen (1–6). The spleens and nodes of mice thymectomized as newborns are deficient in cells capable of exercising graft *versus* host activity in F_1 hybrid mice; conversely, neonatally thymectomized F_1 hybrids are much more vulnerable to immunologic attack by injected parent strain cells (1, 2). The immunologic defect of these animals is almost invariably accompanied by a wasting syndrome, characterized by growth failure, physiologic decline, and early death (1, 2).

The immunologic deficit and growth failure of neonatally thymectomized mice may be prevented by several procedures performed during the first 3 weeks of life, such as grafting of newborn or adult syngeneic or allogeneic thymus (7, 8), or injection of adult syngeneic spleen or lymph node cells (8–10). Partial reconstitution of cellular and immunologic characteristics may also be achieved by intraperitoneal implantation of millipore diffusion chambers containing thymic tissue, provided the graft is made early in life (11, 12).

More recent studies have shown that restoration is possible with spleen cells

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from donors differing from recipients at minor (non-H-2) histocompatibility loci (10), and that immunologic reconstitution is also possible with syngeneic or allogeneic, adult or newborn thymus cells (6, 13).

It is the purpose of this communication to present comprehensive studies involving efforts to reconstitute neonatally thymectomized mice with fully dispersed cells from syngeneic or allogeneic spleen or thymus, extensions of earlier studies. Further, we will report attempts to reconstitute thymectomized mice with spleen grafts from newborn or older donors of the same or other strains, and compare their effectiveness with that of thymic grafts. Finally, extensions of preliminary efforts to *reverse* the wasting process when it is already established in neonatally thymectomized mice will be presented. These results show that the wasting syndrome is regularly a completely reversible process if the mice are given enough thymus or spleen cells which are themselves tolerated by the recipient host.

Materials and Methods

Mice.—Inbred mice of the C_3H/Bi, CE, CBA, A, and BALB/c strains, and (A × C57BL/1)F_1, (A × C_3H)F_1, (C_3H × C57BL/1)F_1, and (C_3H × DBA/2)F_1 hybrids were used. Mice of these strains were separated from the mouse colony of the late Dr. John J. Bittner in 1956, and have been maintained in our laboratories by rigorous inbreeding since that time. The details of housing and care of the animals were given in an earlier paper (1).

Surgical Procedures.—Thymectomies were performed by the technique of Sjodin *et al.* (15). In sham operations, the thorax was opened but the thymus left intact.

Thymus grafts were taken from newborn or older donors, sliced into three or four pieces, and introduced into the subcutaneous tissue of the back of the recipients. If the donor was newborn, the entire thymus was grafted; if older, an equivalent amount of thymus tissue was used. In other experiments splenic tissue was grafted in a similar manner; again, the entire spleen was used if the donor was newborn, and the equivalent of a newborn spleen (approximately 10 to 25 mg of tissue) if the donor was an older animal.

Skin grafts involved the placement of full thickness abdominal skin on a prepared site on the back of the recipient by the technique routinely employed in this laboratory (16). The grafts are turned 180° for ease of identification.

Cell Suspensions.—Suspensions of cells from thymus or spleen were prepared in Ringer's lactate saline solution, using a glass Potter-Elvehjem homogenizer with a loose fitting pestle. Further dissociation of the cells was accomplished by passing the suspension gently in and out of a 27 gauge and then a 30 gauge needle. Cell counts were made, and dilutions prepared so that 25 million nucleated cells were contained in 0.1 ml. Viability studies were performed using carmine and trypan blue staining; viability ranged upward from 90 per cent. All cell injections were intraperitoneal.

Observation.—All animals were observed at least every other day, and weights and clinical characteristics recorded.

Graft Versus Host Assay of Simonsen (17).—This assay involves an assessment of the spleen enlargement in young F_1 hybrid recipients of 30 million spleen cells from one of the parent strains injected intraperitoneally. Litters of 6 to 8, 8-day-old animals of an appropriate hybrid are divided into 3 groups of 2 or 3 mice each. At least 2 mice of each litter are prepared as: (*a*) experimental group, receiving cells from the animals whose immunologic competence is to be assessed; (*b*) negative controls, receiving syngeneic cells; and (*c*) positive controls, receiving

cells from intact or sham-operated mice of the same strain as (*a*). Eight days after cell administration, the hybrids are sacrificed, their body and spleen weights determined, and the relative spleen weight (mg/100 gm body weight) calculated. Finally, the "spleen index" is determined by dividing the mean relative spleen weight of the experimental group by the mean relative spleen weight of the negative controls.

Discriminating Assay of Cell Chimerism.—This assay (18) was employed to ascertain the immunogenetic composition of peripheral lymphoid tissues of mice grafted with allogeneic spleen or thymus, or treated with allogeneic spleen or thymus cells. As in the graft *versus* host assay, spleen enlargement is assessed in young F_1 hybrid recipients of cells from the presumed chimera, but different hybrids are used for host and donor components. Usually one parent strain of the two hybrids will be the same, a strain differing from both the host and donor strains at the H-2 histocompatibility locus. The other parent is donor or host strain depending on the component being assayed. The cell dosage is 30 million administered intraperitoneally. In this assay, the negative controls are prepared by administering to the F_1 hybrid cells from the component of the chimera which will presumably be rejected by the hybrid, *e.g.* A cells into ($C_3H \times$ C57BL/1)F_1 hybrids. The positive controls are given cells from adult animals of the strain expected to be accepted by and react against the hybrid; *e.g.*, A cells into (A $\times$ C57BL/1)F_1. The relative spleen weights (mg/100 gm body weight) are determined at sacrifice of the hybrids 8 days later, and spleen indexes calculated. The experimental spleen index is calculated by dividing the relative spleen weight of the experimental group by that of the negative controls.

Hematology.—Total and differential white cell counts were performed on tail vein blood of 8- to 10-week-old experimental and control mice of the groups involved in reconstitution experiments, and of 16- to 18-week-old mice in the groups studied for reversal of wasting disease. These mice were kept under conditions in which the colony is light from 6 a.m. to 6 p.m., and dark from 6 p.m. to 6 a.m. Blood for lymphocyte counts was drawn between 9 a.m. and 12 noon.

Morphologic Studies.—Groups of mice were sacrificed at 90 to 100 days of age in the reconstitution experiments and at 130 to 150 days of age in the reversal of wasting studies. The thymic area, spleen, axillary lymph nodes, Peyer's patches, and liver were fixed in 10 per cent formalin, cut, and stained as routine with hematoxylin and eosin.

Autopsy.—In all neonatally thymectomized animals sacrificed for graft *versus* host assays or for morphologic study, the absence of mediastinal thymic tissue was verified under the dissecting microscope and by microscopic examination of the mediastinal contents. In any animal bearing a thymus or spleen graft at sacrifice or at death from wasting disease, the status of the graft was also ascertained.

RESULTS

Effect of Administration of Fully Dispersed Syngeneic Thymus Cells to Neonatally Thymectomized Mice.—Neonatally thymectomized C_3H and A strain mice were injected intraperitoneally with either a single dose of 100 million syngeneic thymus cells or with 2 to 4 inocula of 50 million thymus cells each, as shown in Table I. The cell donors were newborn or 2 months old. Restoration was evaluated by body weight, survival to 120 days of age, and capacity of the recipient's spleen cells, at 6 to 9 weeks of age, to induce graft *versus* host reactions as assessed by the method of Simonsen (17).

Text-figs. 1 and 2 indicate that C_3H and A strain mice thymectomized at birth regularly showed growth failure and succumbed between the 27th and

89th day of life. On the other hand, the thymectomized mice receiving syngeneic thymus cells, with few exceptions, showed steady weight increase and prolonged survival (Table I).

Our prior studies, reported elsewhere (13), indicated that the immunologic reactivity of neonatally thymectomized mice, as revealed by capacity to exercise graft *versus* host reactions, could be restored by injections of 100 to 200 million syngeneic thymus cells during the first 15 days of life.

TABLE I

Survival of Neonatally Thymectomized Mice Treated with Fully Dispersed Syngeneic Thymus Cells

Recipients	Age of cell donors	Age of recipients at treatment	Total No. of thymus cells (million)	Survivors at 120 days*
	months	*days*		
Neonatally thymectomized C_3H	2	2, 7	100	6/10
Neonatally thymectomized C_3H	2	8, 11, 13, 15	200	11/14‡
Neonatally thymectomized C_3H	Newborn	15	100	7/9
Neonatally thymectomized C_3H	—	—	—	0/15§
Intact C_3H	—	—	—	19/20
Neonatally thymectomized A	2	5	100	5/6
Neonatally thymectomized A	—	—	—	0/12§
Intact A	—	—	—	18/20

* Most reconstituted animals surviving at 120 days were killed and examined for thymic remnants.

‡ Four reconstituted survivors appeared normal when sacrificed at 14 months of age; no thymic remnants were found.

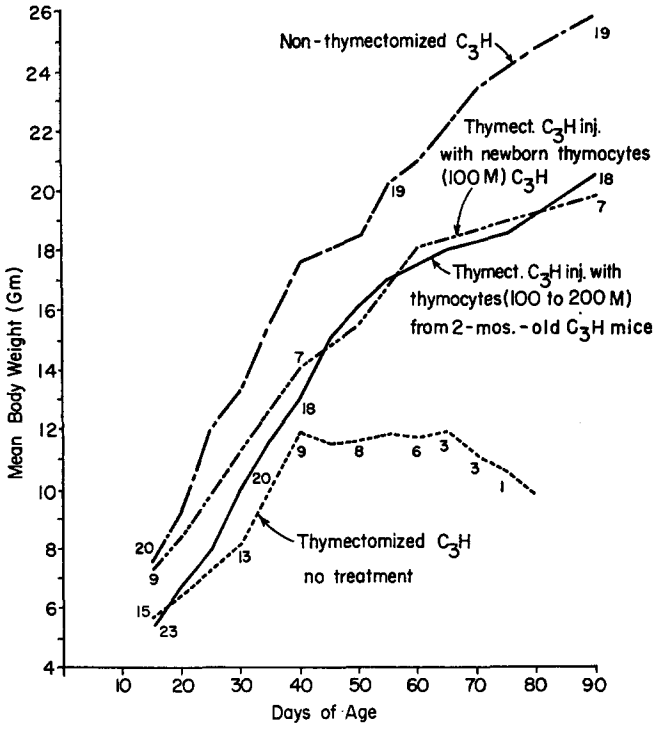
§ All animals died of wasting disease before 80 days of age.

Thus, it may be concluded that neonatally thymectomized mice may be almost completely restored, with respect to growth, longevity, and immunologic capacity, by injection of sufficient numbers of fully dispersed syngeneic thymus cells. Such reconstitution clearly does not require the structural integrity of the thymus itself.

Effect of Administration of Fully Dispersed Allogeneic Thymus Cells to Neonatally Thymectomized Mice.—In these experiments (Table II), the neonatally thymectomized hosts were again of the A and C_3H strains; the donors for the C_3H recipients were newborn or 2-month-old A or (A $\times$ C_3H)F_1 animals, or 2-month-old CBA mice. To distinguish the hybrid, we have used the term "hemiallogeneic." The cell dosage varied from 100 to 400 million cells, and the criteria of reconstitution included growth, survival, and reactivity in the graft *versus* host assay. As a further test of immunologic competence, cell-injected

animals were grafted with skin from the cell donor strain as well as a third allogeneic strain.

Text-fig. 2 and Table II document the growth and survival of the thymectomized A strain mice given C_3H thymus cells. Most of these animals did not

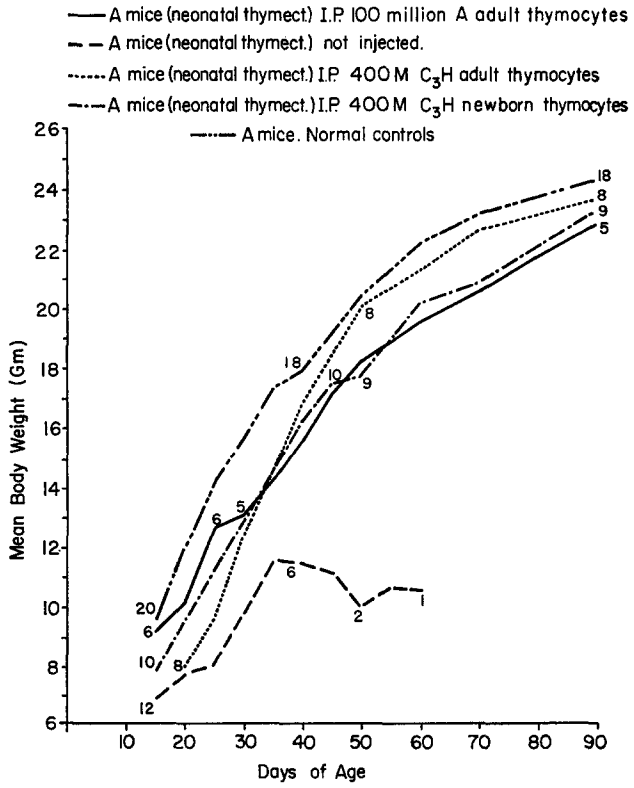


TEXT-FIG. 1. Growth curves of neonatally thymectomized C_3H mice treated with newborn or adult syngeneic thymus cells, compared with those of intact C_3H mice and thymectomized mice given no further treatment. All the points on the curves are means; the numbers on the curves indicate the number of surviving animals at that age. All three forms of treatment averted the wasting disease. It will be seen from the figure that neonatally thymectomized C_3H mice given syngeneic thymus cells from newborn or 2-month-old donors intraperitoneally show growth curves approaching those of normal C_3H animals. Thymus cells expressed in millions (M).

develop wasting disease and survived to 120 days of age. By contrast, most of the thymectomized C_3H mice treated with A strain adult or newborn thymus cells developed an accelerated wasting syndrome and died earlier than most untreated neonatally thymectomized C_3H animals.

When neonatally thymectomized C_3H mice were treated with thymus cells from adult or newborn $(A \times C_3H)F_1$ hybrid donors or from adult CBA donors

(a strain differing from the C_3H strain at weak histocompatibility loci), they showed normal growth well beyond 90 days (Text-fig. 3) and survived to 120 days of age and beyond (Table II).



TEXT-FIG. 2. Growth curves of neonatally thymectomized A mice treated with syngeneic adult thymus cells, or with either adult or newborn C_3H thymus cells, compared with those of intact mice and untreated neonatally thymectomized animals of the same strain. All the points on the curves are means; the numbers on the curves indicate the number of surviving animals at that age. All three treated groups maintained normal growth and had low mortality. Thymus cells expressed in millions (M).

Previous investigations (13) showed that the spleen cell population of thymectomized A strain mice, treated with 400 million C_3H adult thymus cells, was of donor immunologic type, as evaluated by the discriminating spleen assay. Additional studies, summarized in Table III, indicated that fully dispersed newborn thymus cells also reconstituted the allogeneic hosts with respect to donor immunologic and histocompatibility characteristics.

The immunogenetic composition of spleen cells of thymectomized C_3H mice

treated with A strain newborn or adult thymus cells was difficult to assess because most of the animals died before 2 months of age, presumably from graft *versus* host reactions induced by the A strain thymocytes. However, as shown in Table III, the discriminating spleen assays performed in the surviving animals of these groups revealed either no significant activity or activity of donor (A) histocompatibility characteristics.

TABLE II

Survival of Neonatally Thymectomized Mice Treated with Fully Dispersed Allogeneic Thymus Cells

Recipients	Thymus cell donors		Age of recipient at treatment	Total No. of Thymus Cells (million)	Survivors at 120 days
	Strain	Age			
		months	*days*		
Neonatally thymectomized C_3H	A	2	15	300	0/8*
Neonatally thymectomized C_3H	A	Newborn	9	200	0/6*
Neonatally thymectomized C_3H	$(A \times C_3H)F_1$	2	5	200	3/5
Neonatally thymectomized C_3H	$(A \times C_3H)F_1$	Newborn	5, 8, 12, 15, 18, 20, 22, 24	400	12/12‡
Neonatally thymectomized C_3H	CBA	2	10	200	3/3
Neonatally thymectomized A	C_3H	2	7, 12	400	8/8‡
Neonatally thymectomized A	C_3H	Newborn	10	100	4/6
Neonatally thymectomized A	C_3H	Newborn	5, 8, 12, 15, 18, 20, 22, 24	400	9/10‡

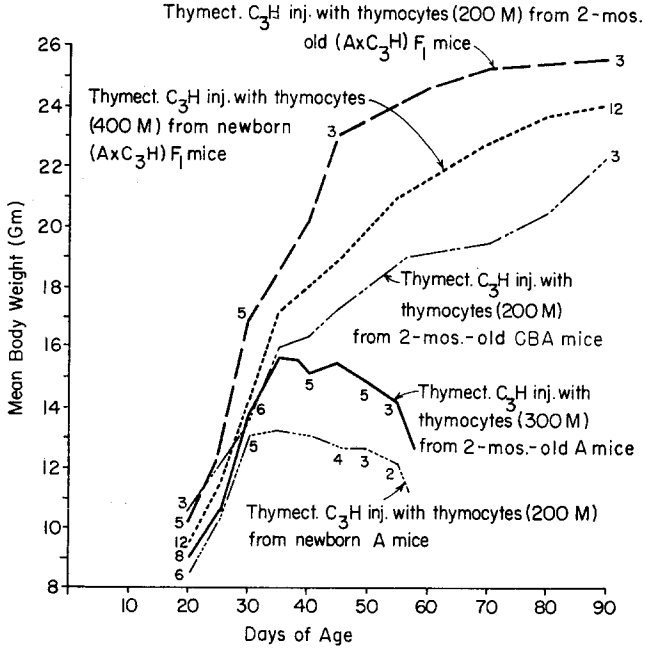
* These animals developed homologous disease and died before 60 days of age.

‡ Two mice from each group were sacrificed at approximately 200 days of age and had no thymic remnants.

The results of allogeneic skin grafting on reconstituted animals were also of interest. Mice of the C_3H strain reconstituted with $(A \times C_3H)F_1$ or CBA thymus cells accepted skin grafts from the cell donor strain or hybrid, but rejected skin from BALB/c donors.

It can, thus, be concluded that hemiallogeneic thymus cells from either adult or newborn donors, and allogeneic thymus cells in certain donor-host combinations, especially those involving weak histocompatibility differences, may provide full restoration of neonatally thymectomized mice as assessed by growth, long-term survival, and immunologic function. In other strain combinations involving more rigorous immunologic barriers, as when A thymus cells are

given to C3H mice, the thymus cells usually accelerate the growth failure and result in early death. Whenever immunologic restoration is provided by foreign thymus cells, the immunologic and histocompatibility characteristics of the spleen cells of the reconstituted animals are those of the donor rather than the recipient strain.



TEXT-FIG. 3. Growth curves of neonatally thymectomized C3H mice treated with newborn or adult, hemiallogeneic or allogeneic thymus cells. All the points on the curves are means; the numbers on the curves indicate the number of surviving animals at that age. While treatment with (A × C3H)F1 and CBA thymus cells averted the wasting syndrome in most of the animals, administration of either newborn or adult A cells resulted in an accelerated wasting disease in the neonatally thymectomized animals. Thymus cells expressed in millions (M).

Effect of Administration of Syngeneic or Allogeneic Adult Spleen Cells to Neonatally Thymectomized Mice.—In this series, attempts were made to reconstitute neonatally thymectomized C3H mice by intraperitoneal injection of 10 million spleen cells from 2-month-old syngeneic donors; 10 or 100 million spleen cells from allogeneic donors, differing at weak or strong histocompatibility loci from the recipients; and finally 10 or 100 million cells from hemiallogeneic donors. Reconstitution was evaluated by growth, survival to 120 days, and the homograft response.

The results of these experiments are shown in Table IV and Text-fig. 4. Thymectomized C3H mice injected with cells from CBA or CE donors (all

TABLE III

Discriminating Spleen Assay of Neonatally Thymectomized Mice Treated with Fully Dispersed Allogeneic Thymus Cells

Thymectomized recipient strain	Thymus cell donors		Total No. of thymus cells* (millions)	Component assayed	Test litters	Range of spleen indexes‡	Mean spleen index	Number of Mice with significant graft *versus* host activity (spleen index > 1.30)
	Strain	Age						
		months						
A	C_3H	Newborn	400	Donor	$(C_3H \times C57BL/1)F_1$	0.87–2.19	1.59	3/4
				Host	$(A \times C57BL/1)F_1$	0.98–1.07	1.00	0/4
C_3H	A	2	300	Donor	$(A \times C57BL/1)F_1$	1.58	1.58	1/1
				Host	$(C_3H \times C57BL/1)F_1$	1.00	1.00	0/1
C_3H	A	Newborn	200	Donor	$(A \times C57BL/1)F_1$	0.98–2.08	1.30	1/5
				Host	$(C_3H \times C57BL/1)F_1$	0.86–1.14	1.03	0/4

* Thymus cells were injected intraperitoneally during the first 4 weeks of life, as shown in Table II.

‡ Spleen indexes were calculated by dividing the relative spleen weight (mg/100 gm body weight) of the experimental group by the relative spleen weight of the negative control group (syngeneic spleen cell injected animals of the same hybrid).

TABLE IV

Effect of Treatment with Adult Spleen Cells on Longevity and Skin Homograft Immunity of Neonatally Thymectomized Mice

Neonatally thymectomized recipient strain	Spleen cell donor strain	No. of cells (million)	Survivors at 120 days*	Skin graft		Skin graft	
				Donor strain	Rate of acceptance‡	Donor strain	Rate of acceptance‡
C_3H	C_3H	10	7/7	—	—	BALB/c	0/5
C_3H	CBA	10	4/6	CBA	4/4§	BALB/c	0/4
C_3H	CBA	100	6/6	CBA	5/5§	BALB/c	0/5
C_3H	CE	10	6/7	CE	6/6§	BALB/c	0/6
C_3H	CE	100	4/6	CE	4/4§	BALB/c	0/4
C_3H	$(A \times C_3H)F_1$	10	7/8	$(A \times C_3H)F_1$	6/6§	BALB/c	0/6
C_3H	$(A \times C_3H)F_1$	100	5/6	$(A \times C_3H)F_1$	5/5§	BALB/c	0/5
C_3H	A	10	0/11	—	—	—	—
C_3H	A	100	0/5	—	—	—	—
A	C_3H	10	5/6	C_3H	3/3	BALB/c	1/3‖
A	C_3H	100	3/3	C_3H	3/3	BALB/c	0/3

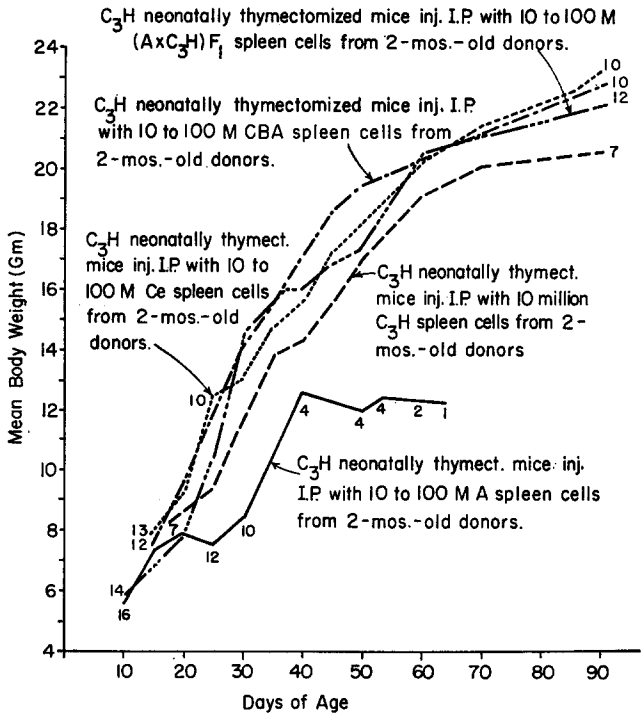
* Most reconstituted survivors were sacrificed at 120 days and examined for thymic remnants.

‡ Number of mice accepting grafts for 30 days or more over the number grafted.

§ Two mice from each group were sacrificed at about 200 days of age; no thymic remnants were found.

‖ This graft was rejected 38 days after application.

H-2^k but differing at weak histocompatibility loci) gained weight normally (Text-fig. 4) and showed a large percentage of survivors at 120 days (Table IV). Similar results were obtained when the spleen cell donors were (A × C_3H)F_1 hybrid animals. Skin grafting studies confirmed the immunologic restoration

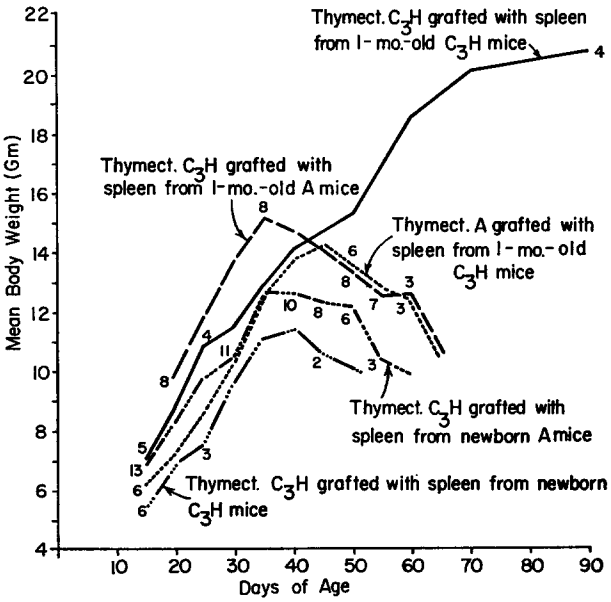


TEXT-FIG. 4. Growth curves of neonatally thymectomized C_3H mice treated with adult spleen cells from syngeneic, hemiallogeneic, or allogeneic donors. All the points on the curves are means; the numbers on the curves indicate the number of surviving animals at that age. All the treatments were successful in averting wasting except injection of adult A strain spleen cells. Spleen cells expressed in millions (M).

of the animals: skin from the strain of the spleen cell donor was consistently accepted and skin from a third strain, BALB/c, regularly rejected (Table IV).

On the other hand, thymectomized C_3H mice treated with A strain spleen cells (an H-2 histocompatibility difference) failed to gain weight and died before 65 days of age. By contrast, neonatally thymectomized A strain mice injected with 10 or 100 million adult C_3H spleen cells survived and were able to accept C_3H skin grafts and to reject BALB/c skin.

Effect of Syngeneic or Allogeneic Spleen Grafts on Neonatally Thymectomized Mice.—In the first series, neonatally thymectomized C_3H mice were grafted subcutaneously at 3 days of age with newborn spleen from A or C_3H donors;



TEXT-FIG. 5. Growth curves of neonatally thymectomized C_3H mice grafted with newborn or adult, syngeneic or allogeneic spleen. All the points on the curves are means; the numbers on the curves indicate the number of surviving animals at that age. Only the grafting of syngeneic spleen from 1-month-old donors averted the wasting disease. By contrast, spleen grafts from newborn syngeneic donors or from adult or newborn allogeneic (A strain) donors failed to prevent wasting disease.

TABLE V

Survival of Neonatally Thymectomized Mice Grafted with Syngeneic or Allogeneic Spleen

Neonatally thymectomized recipient strain	Spleen graft strain	Donor age	Recipient age at grafting	Survivors at 120 days
		months	*Days*	
C_3H	C_3H	1	2	4/5*
C_3H	C_3H	Newborn	2	0/6‡
C_3H	A	1	2	0/8‡
C_3H	A	Newborn	2	0/13‡
A	C_3H	1	15	0/6‡

* Survivors were sacrificed at 120 days and examined for thymic remnants.

‡ All the mice in these groups died by 65 days of age; the spleen grafts were intact in all instances.

in the second, thymectomized A strain mice were grafted at 15 days of age with spleen from 1-month-old C_3H animals.

The mean body weights of these groups of mice, illustrated in Text-fig. 5, show that most of the animals grafted with either syngeneic newborn or allo-

geneic adult spleen tissue began to lose weight approximately 30 days after grafting and died by 65 days of age. On the other hand, C_3H mice thymectomized as newborns and grafted with spleen from 1-month-old syngeneic mice gained weight at a normal rate and survived (Table V).

Figs. 1 to 6 illustrate spleen grafts recovered from neonatally thymectomized mice sacrificed at 6 weeks of age. The adult grafts (Figs. 1 to 3), which reconstituted the syngeneic hosts, showed well developed lymphoid cells and typical lymphoid organization. However, the newborn syngeneic grafts, composed primarily of reticulum, myeloid, and erythroid cells when grafted into the thymectomized hosts at 3 days of age, remained immature organs and did not show lymphoid maturation 5½ weeks after placement (Figs. 4 to 6). The recipients of these newborn spleen grafts were not reconstituted.

TABLE VI

Graft Versus Host Assay in (C_3H × DBA)F_1 Hybrid Recipients of Spleen Cells from Neonatally Thymectomized Mice Grafted with Syngeneic Spleen

Group	Age of spleen graft donor	Range of spleen indexes*	Mean spleen index	No. of Mice with significant graft *vs.* host activity (spleen index > 1.30)
	months			
Thymectomized C_3H	1	1.91–2.52	2.21	2/2
Thymectomized C_3H	Newborn	0.83–1.14	0.97	0/3
Intact C_3H	—	2.19–3.52	2.96	5/5

* Spleen indexes were calculated by dividing the relative spleen weight (mg/100 gm body weight) of the experimental group by the relative spleen weight of the negative control group (syngeneic spleen cell-injected animals).

Immunogenetic Analysis of Spleen Cells of Spleen-Grafted Neonatally Thymectomized Mice.—These studies were undertaken to ascertain the immunologic activity in graft versus host reactions of host spleen cells from neonatally thymectomized C_3H mice bearing syngeneic spleen grafts, and the immunogenetic composition of host spleen cells from allogeneic (A) spleen-bearing C_3H animals. These are different groups from those discussed in the preceding section; however, the procedures were the same.

The syngeneic spleen-grafted animals were sacrificed at 5 to 8 weeks, and suspensions of spleen cells prepared for injection into 8-day-old (C_3H × DBA/2)F_1 hybrids. Each animal received 30 million cells. Syngeneic hybrid cells were administered to the negative controls and adult C_3H cells to the positive controls. Splenomegaly was assessed 8 days later, and spleen indexes calculated as shown in Table VI. Adult C_3H spleen cells resulted in spleen indexes of 2.00 or more, indicative of vigorous graft *versus* host activity. Similarly, the thymectomized mice grafted in the newborn period with adult

syngeneic spleen showed significant restoration of immunologic function by this criterion. By contrast, no restoration of graft *versus* host activity resulted from syngeneic newborn spleen grafts.

In the groups of thymectomized C_3H mice grafted with A strain spleen from newborn or 1-month-old donors, the immunogenetic characteristics of cells of the host spleen were determined by the discriminating spleen assay, testing the host component in the $(C_3H \times C57BL/1)F_1$ hybrid and the donor component in the $(A \times C57BL/1)F_1$ hybrid. As shown in Table VII, there was no restoration of either donor or host reactivity when newborn spleen transplants were used. However, thymectomized recipients of allogeneic spleen grafts from

TABLE VII

Discriminating Spleen Assay of Neonatally Thymectomized Mice Grafted with Allogeneic Spleen

Recipient	Spleen graft donors		Component assayed	Test litters	Range of spleen indexes*	Mean spleen index	No. of Mice with significant graft *vs* host activity (spleen index > 1.30)
	Strain	Age					
		months					
Thymectomized C_3H	A	1	C_3H (host)	$(C_3H \times C57BL/1)F_1$	1.00–1.14	1.07	0/2
			A (donor)	$(A \times C57BL/1)F_1$	1.91–2.01	1.96	2/2
Thymectomized C_3H	A	Newborn	C_3H (host)	$(C_3H \times C57BL/1)F_1$	0.94–1.14	1.03	0/6
			A (donor)	$(A \times C57BL/1)F_1$	0.97–1.03	1.00	0/6

* Spleen indexes were calculated by dividing the relative spleen weight (mg/100 gm body weight) of the experimental group by the relative spleen weight of the negative control group (syngeneic spleen cell-injected animals).

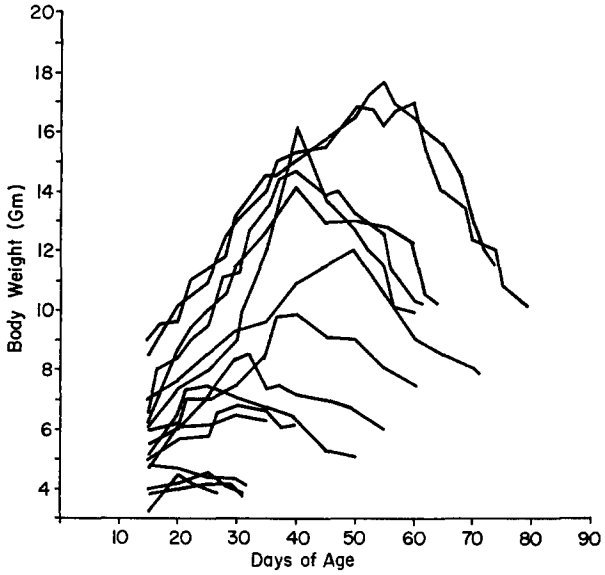
1-month-old donors showed significant immunologic reconstitution, entirely attributable to donor immunogenetic characteristics.

These results indicate that spleen grafts from syngeneic, immunologically mature animals, possessing mature lymphoid structure and organization, will provide reconstitution of neonatally thymectomized mice. By contrast, spleen grafts from newborn (immunologically immature) syngeneic donors, possessing little lymphoid tissue, remain immature in the thymectomized recipient and do not provide reconstitution of immunologic function or of capacity to grow and survive under the conditions prevailing in the laboratory. When reconstitution of neonatally thymectomized mice is achieved by allogeneic spleen transplants, the immunologic reconstitution is with respect to cells of the donor and not the host immunohistocompatibility characteristics.

Reversal of Wasting Disease in Thymectomized Mice by Injection of Syngeneic Spleen or Thymus Cell Suspensions.—In these studies the administration of

syngeneic spleen or thymus cells was withheld until the neonatally thymectomized C_3H recipients had developed wasting disease. Body weights were recorded every 3 days. Text-fig. 6 shows the body weight changes observed in 15 C_3H mice thymectomized at birth; based on these data, the onset of wasting was considered to be the day of highest weight attained.

The animals selected for treatment had reached a maximum weight of at least 10 gm, followed by loss of 10 to 30 per cent of body weight, or by a failure of weight gain of at least 10 days' duration, and had other clinical signs of



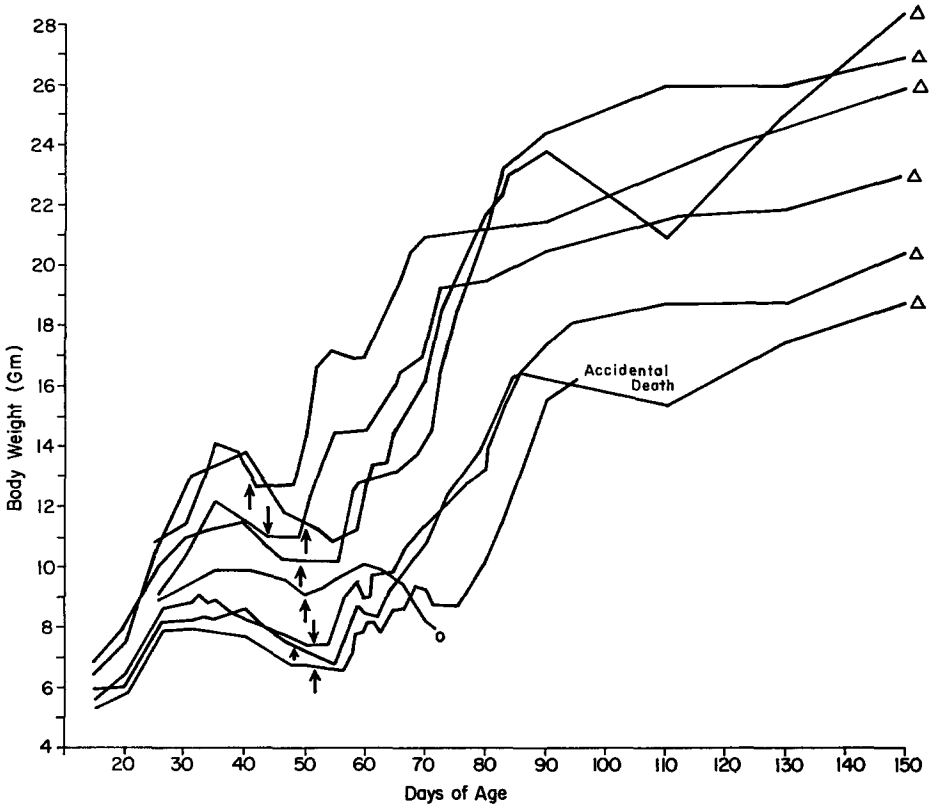
TEXT-FIG. 6. Individual weight curves of 15 neonatally thymectomized C_3H mice, showing the variability in the physiologic decline of these animals, resulting in death of all the animals before 90 days of age.

wasting disease. At this time, when the wasting animals were 37 to 47 days old, each was injected intraperitoneally with 200 million syngeneic spleen or thymus cells. Reversal of the wasting process was considered to have been achieved if the treated mice showed a return to normal body growth and were healthy in appearance 60 days later.

Eight of 9 spleen cell–treated mice began to gain weight 5 to 12 days after treatment and reached the maximum pretreatment weight 6 to 20 days after cell administration. Each of these animals has continued to grow normally to the time of writing and is in good condition 1 year after treatment. Reversal of wasting disease was also observed in 3 of 10 thymus cell–injected animals (14).

The immunologic capacity of spleen cells from some of these animals was

assessed by the graft *versus* host assay. The animals which recovered from the wasting disease were also restored immunologically, as judged by graft *versus* host activity in appropriate F_1 hybrid animals (14).



TEXT-FIG. 7. Individual weight curves of 8 neonatally thymectomized C_3H mice injected with hemiallogeneic (A × C_3H)F_1 spleen cells after onset of the wasting syndrome. The arrow indicates the time of cell injection in each instance. One animal died of wasting disease. Seven showed arrest of weight loss and return to normal weight gain; one of these died accidentally at about 90 days of age, and the others were alive and in good condition clinically at 150 days when they were sacrificed. None had thymus remnants.

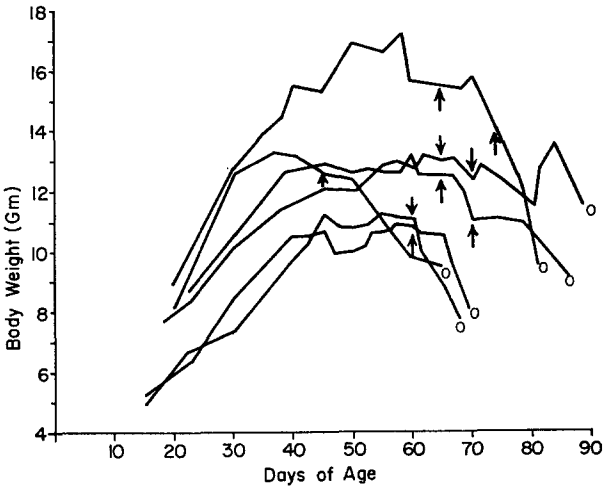
Reversal of Wasting Disease in Thymectomized Mice by Injection of Allogeneic Spleen Cells.—A and C_3H mice were thymectomized as newborns, and their weights recorded every 3 days. They reached their maximum weight, from 7.0 to 17.2 gm, at ages ranging from 25 to 62 days. The allogeneic spleen cell dosage was 200 million cells, administered when the animals had clinical signs of wasting and had either stopped gaining or lost weight. The time of treatment bore a variable relationship to peak weight, ranging from failure to gain for 1

TABLE VIII

Immunologic Reconstitution of Mice with Postthymectomy Wasting Disease after Treatment with Allogeneic or Hemiallogeneic Spleen Cells

Neonatally thymectomized recipients	Cell donor strain	Treatment*	Survivors 120 days after treatment	Skin graft		Skin graft	
				Donor	Rate of acceptance‡	Donor	Rate of acceptance‡
C3H	(A × C3H)F1	200 × 10⁶ adult spleen cells	7/8§	(A × C3H)F1	6/6	BALB/c	0/6
C3H	CE	200 × 10⁶ adult spleen cells	4/6§	CE	3/3	BALB/c	0/3
C3H	CBA	200 × 10⁶ adult spleen cells	2/3	CBA	2/2	BALB/c	0/2
C3H	—	—	0/31‖	—	—	—	—

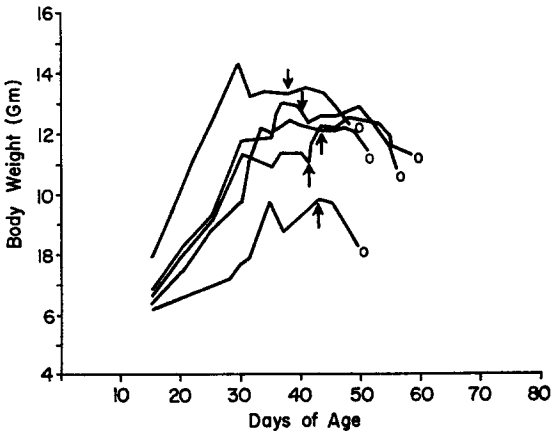
* Cells were administered 5 to 20 days after onset of wasting.
‡ Number of animals accepting the graft for 30 days or more.
§ One of the reconstituted survivors died accidentally 45 days after treatment.
‖ All mice in this group died of wasting disease before 80 days of age.



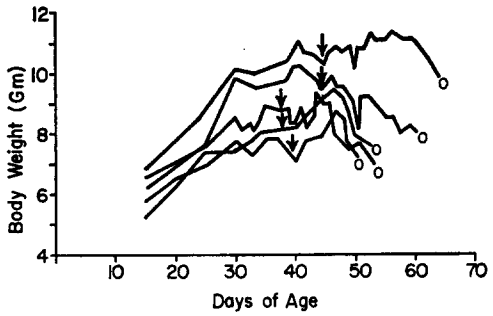
TEXT-FIG. 8. Individual weight curves of 6 neonatally thymectomized A mice treated with 200 million C3H spleen cells after onset of wasting. The decline of the animals was not affected by the treatment, and all had died by 90 days of age.

to 15 days, to loss of 5 to 28 per cent of peak body weight over periods of 5 to 26 days. Again, the criterion of reversal was a return to normal weight gain and prolonged survival.

Text-fig. 7 shows the reversal of wasting in C3H mice receiving spleen cells from 2-month-old (A × C3H)F1 hybrid donors. Weight gain was restored, and most of the animals survived in good condition to 150 days of age. In additional



TEXT-FIG. 9. Individual weight curves of 5 neonatally thymectomized C_3H mice grafted with syngeneic newborn thymus after onset of wasting, as indicated by arrows. None of the animals showed clinical improvement; all died before 70 days of age. The thymus grafts were in good condition at the time of death.



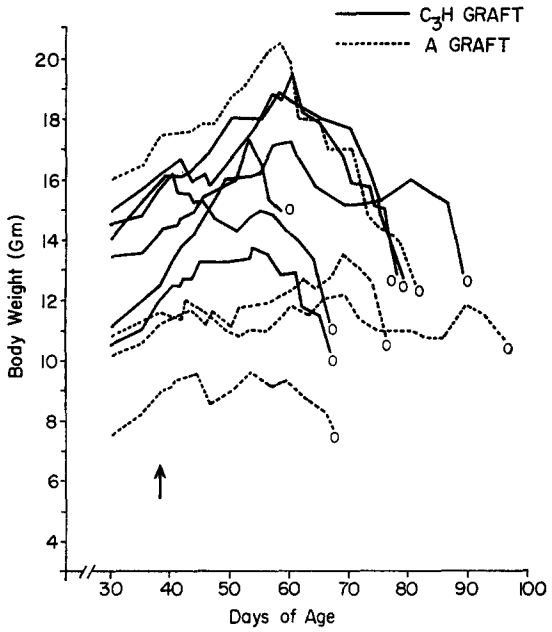
TEXT-FIG. 10. Individual weight curves of 5 neonatally thymectomized C_3H mice grafted with syngeneic adult thymus after onset of wasting, as indicated by arrows. None of the animals recovered, and all had died before 70 days of age. The thymus grafts were in good condition at the time of death.

experiments, we found that neonatally thymectomized C_3H mice which have already developed the wasting syndrome can be fully restored by injection of spleen cells from CBA or CE animals, strains differing from the C_3H strain at minor histocompatibility loci. The survival data on these animals are summarized in Table VIII.

When neonatally thymectomized A strain mice with overt signs of wasting disease were treated with spleen cells from C_3H donors (an H-2 histocompatibility difference), the wasting disease progressed and the animals died early (Text-fig. 8).

Immunologic capacity of the animals that recovered from wasting disease following hemiallogeneic or allogeneic spleen cell administration was tested by applying a skin graft from a third strain, BALB/c, as shown in Table VIII. All these grafts were rejected, while grafts from the strain of the cell donors were accepted in all instances.

These observations establish that the wasting disease of neonatally thymectomized mice, whatever its basis, may be reversed by treatment with syngeneic,



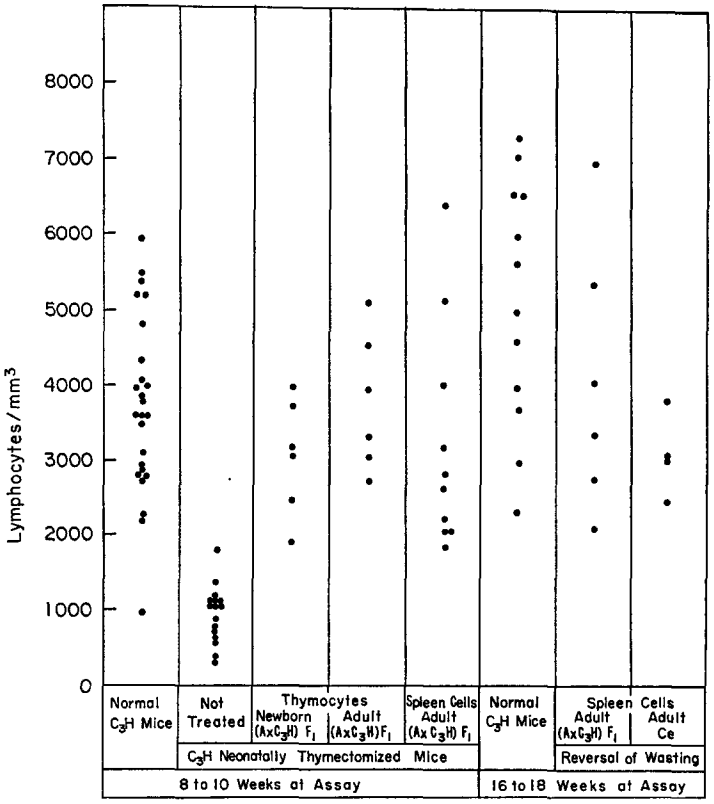
TEXT-FIG. 11. Individual weight curves of 10 neonatally thymectomized C_3H mice, grafted at 38 days of age, with either A or C_3H newborn thymus. Although none of the animals had clinical wasting disease at the time of grafting, all went on to develop the typical runting and wasting syndrome, and died before 100 days of age with thymus grafts intact.

allogeneic, or hemiallogeneic spleen cells. Treatment of wasting animals with allogeneic cells, where donor and recipient differ at strong histocompatibility barriers, does not reverse the progressive growth failure or prolong life.

Treatment of Postthymectomy Wasting Disease by Thymus Grafts.—The general experience, in our own and other laboratories, has been that thymus grafts are of no benefit to mice that have already developed wasting disease.

Text-figs. 9 and 10 show the growth curves of neonatally thymectomized C_3H mice grafted with syngeneic newborn (Text-fig. 9) or 2-month-old (Text-fig. 10) thymus when signs of wasting were already present. These data reveal no significant weight gain when compared with control growth curves; clinically, the animals showed no other benefits from the grafts. All died early.

In another group of C_3H animals, thymectomized as newborns, syngeneic or allogeneic (A) grafts were applied at 38 days, late in the prewasting period but before signs of the disease had appeared. Text-fig. 11 illustrates the onset of the wasting syndrome and the early death despite the thymic grafts.



TEXT-FIG. 12. Lymphocyte levels of individual C_3H mice: normal, thymectomized, and reconstituted with hemiallogeneic thymus or spleen cells. All were 8 to 10 weeks of age at the time of assay. Also illustrated is a comparison of lymphocyte counts of normal C_3H animals with those of two groups of animals whose wasting disease was reversed by administration of spleen cells. The counts were done at 16 to 18 weeks of age when the animals were well clinically. The immunologic reconstitution of all these groups was paralleled by the restoration of circulating lymphocyte levels to the normal range in most of the animals.

The experience in both of these groups stands in striking contrast with that of neonatally thymectomized C_3H animals grafted at 7 days of age with newborn syngeneic or hemiallogeneic thymus. The latter animals did not develop wasting and survived normally.

In all of these grafted animals, the thymus tissue was recovered at autopsy

TABLE IX

Reconstitution of Lymphoid Tissues of Neonatally Thymectomized C_3H Mice Treated before and after the Onset of Wasting Disease

Treatment	Tissue examined	No. of Mice	No. of Mice with lymphoid population		
			Normal	Moderately depleted	Markedly depleted
Spleen graft from C_3H 4-week-old mice	Spleen	3	2	1	0
	Lymph node	0			
	Peyer's patch	3	1	1	1
Spleen graft from C_3H newborn mice	Spleen	5	0	1	4
	Lymph node	0			
	Peyer's patch	2	0	1	1
100×10^6 spleen cells from 8 to 10-week-old $(A \times C_3H)F_1$ mice	Spleen	5	3	1	1
	Lymph node	4	1	2	1
	Peyer's patch	5	3	1	1
200×10^6 thymus cells from 8 to 10-week-old $(A \times C_3H)F_1$ mice	Spleen	3	0	2	1
	Lymph node	3	2	1	0
	Peyer's patch	3	1	1	1
400×10^6 thymus cells from newborn $(A \times C_3H)F_1$ mice	Spleen	2	1	1	0
	Lymph node	3	1	2	0
	Peyer's patch	3	2	1	0
Reversal of wasting 200×10^6 spleen cells from 8 to 10-week-old $(A \times C_3H)F_1$ mice	Spleen	5	2	2	1
	Lymph node	5	2	3	0
	Peyer's patch	5	1	3	1
Reversal of wasting 200×10^6 spleen cells from 8 to 10-week-old CE mice	Spleen	3	1	1	1
	Lymph node	3	1	2	0
	Peyer's patch	3	1	2	0
No treatment	Spleen	20	0	15	5
	Lymph node	20	0	10	10
	Peyer's patch	20	0	1	19

and shown to be vascularized, normal appearing tissue, not significantly different from the curative grafts placed in younger animals before growth failure had occurred.

Lymphocyte Counts.—Text-fig. 12 illustrates the absolute lymphocyte counts in the peripheral blood of 8- to 10-week-old experimental and control mice. Twenty-four of 25 normal C_3H mice had absolute lymphocyte counts above 2000 per mm^3, and all 16 neonatally thymectomized animals of the same strain had counts below 2000 per mm^3. Almost all of the mice protected from wasting by injection of hemiallogeneic spleen or thymus cells had counts

above 2000 per mm^3, as did the C_3H animals whose wasting disease was reversed by hemiallogeneic or allogeneic spleen cell administration. The counts of the latter groups were performed at 16 to 18 weeks of age.

Histologic Studies.—The histology of the spleen, lymph nodes, and Peyer's patches was evaluated in randomly selected animals of the several reconstituted groups at 90 to 100 days of age, except for the animals whose wasting disease was reversed by cell administration, sacrificed at 130 to 150 days. Comparable tissues were taken from long-term survivors among the neonatally thymectomized animals not given further treatment, as well as from a group of animals unsuccessfully treated with newborn syngeneic spleen grafts. In an effort to provide objective data, the lymphoid population of the tissues was classified, without knowledge of their source, as normal, moderately depleted, or markedly depleted. The follicular structure was also assessed.

In neonatally thymectomized C_3H mice the lymphoid tissues show either marked or moderate depletion of lymphocytes, most extreme in the Peyer's patches in our series and least so in the spleen, as shown in Table IX. The degree of deficiency of follicular structure was variable, but tended to be closely related to the deficiency in the total number of lymphoid cells present in the lymphoid tissue.

Evidence of some degree of deficiency of total numbers of tissue lymphocytes and lack of normal follicle formation were still noted in some neonatally thymectomized mice reconstituted by intraperitoneal injection of spleen or thymus cells, or by subcutaneous spleen grafts, but in most of the animals these tissues showed evidence of restoration of varying degree (Table IX), and some even had a very normal morphologic structure. In the neonatally thymectomized animals grafted with newborn syngeneic spleen, there was no evidence of significant restoration of the lymphoid tissues.

Figs. 7 to 14 illustrate the morphologic deficiencies of the peripheral lymphoid tissues of neonatally thymectomized mice, and reflect the high degree of restoration of the morphology of these tissues achieved in some animals by administration of thymus or spleen cells alone even in mice that had already developed the wasting syndrome before treatment was begun.

DISCUSSION

The observations presented here are of interest from several points of view. First, we have shown that procedures which reestablish the lymphoid cellularity of the peripheral lymphoid tissue of neonatally thymectomized mice also reconstitute them immunologically and permit them to grow and develop normally. Procedures found to be effective included intraperitoneal injection of adult or newborn thymus cells, or of adult spleen cells, from syngeneic, hemiallogeneic, and allogeneic donors; and early grafting of adult spleen or of adult or newborn thymus.

As in earlier studies (7, 8, 19, 20), thymus grafts provided reconstitution

only if performed well before onset of the wasting syndrome, while thymus and spleen cells restored older animals even those with well advanced wasting disease (8, 14). Since thymus grafts apparently function, at least in part, by humoral, presumably hormonal, means (11, 12) these findings suggest that the spleen, lymph nodes, and other peripheral tissues become refractory to the effects of the hormone, perhaps as a result of failure of proliferation or death of potentially competent lymphoid cells seeded earlier from the thymus to the periphery, or because of loss, in the absence of hormonal support, of developmental potential of reticular cells.

By contrast, reconstitution with dispersed thymus or spleen cells permitted establishment in the peripheral lymphoid organs of an apparently self-sustaining population of lymphoid cells. These were always of donor histocompatibility characteristics when allogeneic donors were employed (8, 13, 14). The success of "late" cell administration in preventing wasting and immunologic inadequacy, and particularly in reversing the wasting syndrome when it is fully manifest, is a strong indication that the essential defect in neonatally thymectomized mice is the deficiency of lymphoid cells in peripheral tissues. Certainly animals reconstituted in the present studies have grown and survived, in some instances for as long as 14 months, possessing normal or near normal immunologic vigor, in the complete absence of a thymus gland.

If wasting disease is a consequence of infection or infestation, or of intoxication from bacterial, viral or fungal flora, as recent studies with germ-free mice suggest, (21, 22), the infection or intoxication is preventable and even completely reversible if the mice are given enough lymphoid cells from syngeneic, hemiallogeneic, or even allogeneic donors. It is important that the lymphoid cells administered be cells incapable of destructive graft *versus* host assault on the thymectomized host. If, as deVries *et al.* (23, 24) have suggested, the runting and wasting of neonatally thymectomized mice is a consequence of an autoimmune process, this destructive process is reversible simply by providing the neonatally thymectomized host with a lymphoid cell population capable of repopulating the peripheral lymphoid organs.

Of interest was the finding that adult spleen grafted into the neonatally thymectomized mice in very early life reconstituted the animals, but that newborn spleen did not. This confirms earlier findings that the newborn spleen in many mouse strains is primarily reticular, myelopoietic, and erythropoietic, and contains few lymphoid cells (1); and the observation of Auerbach (25) that the embryonic mouse spleen is incapable of lymphoid development *in vitro* in the absence of thymus.

Also worthy of attention is the contrast in the results of reconstitution efforts in C_3H and A mice. Neonatally thymectomized A mice were regularly salvaged after injection of C_3H newborn or adult thymus cells, despite the existence of an H-2 histocompatibility barrier between the strains. However, neonatally thymectomized C_3H mice were rarely saved from wasting disease

by injection of neonatal or adult A thymus cells. This suggests that, even at birth, A strain thymus cells are capable of immunologic assault on C_3H tissues, while C_3H thymus cells have relatively weak capacity to react against A strain recipients. The results of this reciprocal reconstitution experiment may contribute to understanding of the relative ease of producing tolerance in neonatal C_3H mice using A cells or antigens from A cells, and the great difficulty of producing immunologic tolerance in newborn A mice with C_3H cells or antigens (26 to 28).

Our morphologic studies show, as have others (1, 2, 7, 9, 19), the deficiency of lymphocytes not only in the peripheral blood but in the lymph nodes, spleen, and Peyer's patches of neonatally thymectomized mice. The restoration achieved by cell administration and spleen or thymus grafting has been illustrated, and is consistent with the concept expressed above, that the essential defect in neonatally thymectomized mice is underdeveloped peripheral lymphoid tissue. Infection, runting, wasting, early death, immunologic inadequacy, and even "autoimmune processes" sometimes observed, seem to us to be consequences of this inadequacy.

Of potential clinical significance is the effectiveness of late administration of thymus or spleen cells contrasted with the ineffectiveness of late thymus grafts. Consequently, in approaching the clinical problems of the thymus-based immunologic deficiencies, such as the Swiss type of agammaglobulinemia, in which lymphopenia, immunologic inadequacy, runting, and wasting seem to be attributable to a vestigial, underdeveloped thymic primordium (29 to 34), or ataxia telangiectasia, in which varying degrees of immunologic deficiency are associated with incomplete lymphoid maturation of the thymus (35), it may be fruitless to transplant thymus. Rather, we would predict from the observations presented here, that a more incisive approach would be to provide these patients with an adequate population of allogeneic thymic or peripheral lymphoid cells, preferably matched to recipient with respect to major histocompatibility characteristics.

SUMMARY

1. Immunologic function, growth, and longevity of neonatally thymectomized mice was restored by intraperitoneal administration of 100 to 400 million syngeneic, hemiallogeneic, or allogeneic thymus cells from newborn or adult donors. Assays of the graft *versus* host capabilities of spleen cells from the animals restored with allogeneic cells showed that their immunologically competent cells are of donor histocompatibility characteristics. Such animals accepted skin grafts from mice of the cell donor strain, but rejected skin from a third strain.

2. Similar results were obtained when the neonatally thymectomized animals were treated with 10 to 100 million syngeneic, hemiallogeneic, or allogeneic cells from adult spleen.

3. In one strain combination, C_3H recipients and A donors, injected thymus

or spleen cells apparently attacked host tissues, since the animals died very early of wasting disease. When this combination was reversed, A strain recipients treated with C_3H cells were reconstituted immunologically and physiologically.

4. Syngeneic or allogeneic adult spleen, grafted in the newborn period, reconstituted neonatally thymectomized mice, but all experiments involving grafting of newborn spleen failed. Immunogenetic analysis of the host spleen cells from two allogeneic spleen-grafted animals previously thymectomized showed that the reconstitution was entirely of donor histocompatibility characteristics.

5. Postthymectomy wasting disease was reversed by administration of 200 million adult syngeneic spleen or thymus cells. Immunologic recovery was confirmed by graft *versus* host assays of the spleens of the recovered animals and by application of allogeneic skin grafts. Some of the animals have been under observation for 42 weeks and appear to be normal.

6. The wasting syndrome in neonatally thymectomized mice was also reversed by injection of 200 million hemiallogeneic or allogeneic spleen cells.

7. Thymus grafts did not reverse wasting disease, whether the donors were adult or newborn, of the same strain or a different one.

8. Spleen, lymph node, and Peyer's patches from representative animals of the reconstituted groups were examined and compared with the tissues of untreated neonatally thymectomized mice and intact animals of the same strain. Tissues of normal cellularity and follicular organization were found in some of the reconstituted animals and also in mice with reversed wasting disease. Extreme deficit of the lymphoid tissues was rare in either group.

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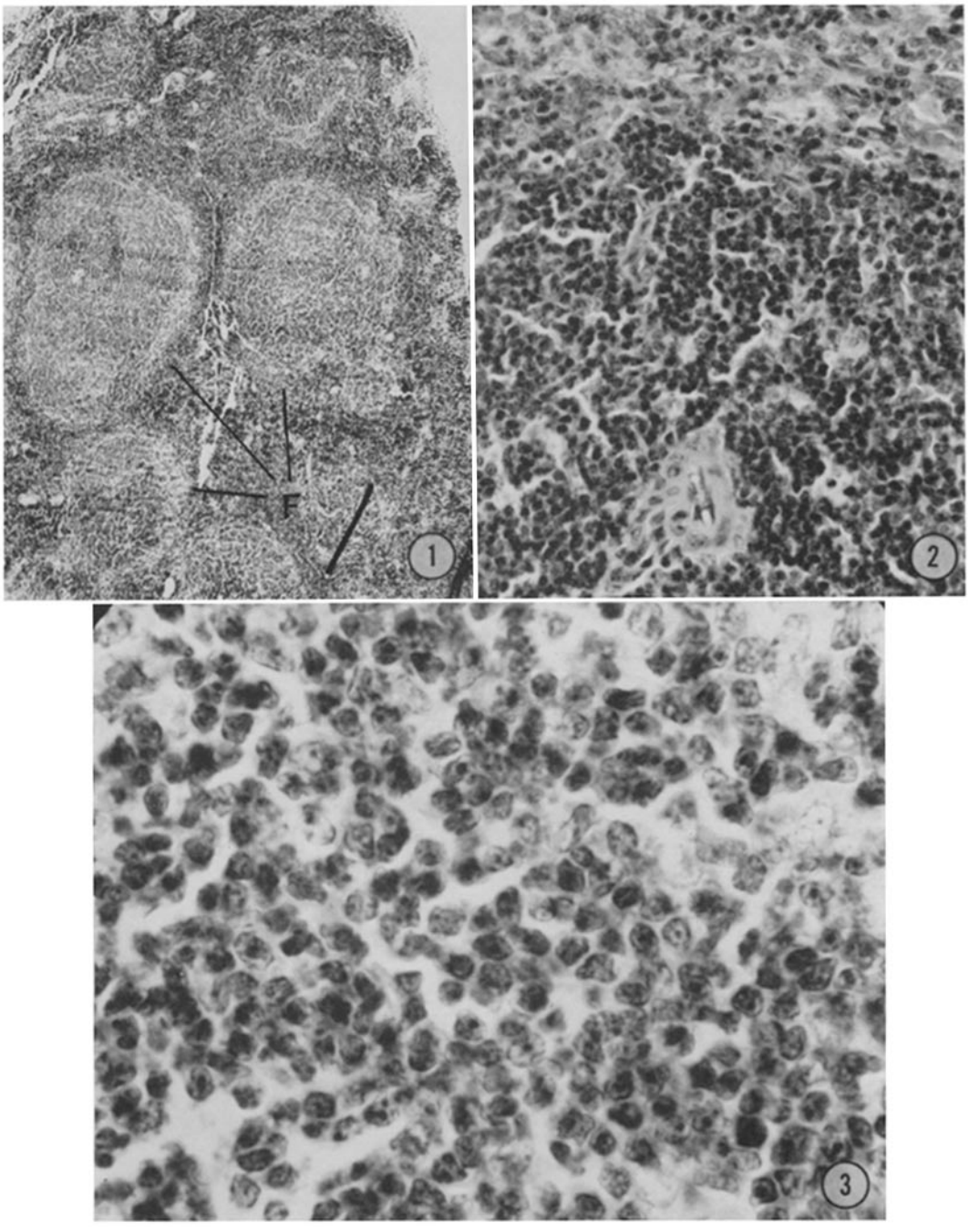
EXPLANATION OF PLATES

PLATE 50

FIG. 1. Low power view of an adult C_3H spleen graft, implanted in a neonatally thymectomized C_3H recipient at 3 days of age, and recovered at 6 weeks. Such grafts maintained their structure and cellularity, and restored immunologic function in the recipients. Note the well developed lymphoid follicular structures (*F*) which make up a large part of the graft. × 40.

FIG. 2. Medium power view of part of a lymphoid follicle of the adult spleen graft in a neonatally thymectomized C_3H recipient. Same tissue as shown in Fig. 1. Note that the follicular accumulation is made up of densely packed lymphocytes scattered among the stromal reticulum cells. × 400.

FIG. 3. High power view of portion of Fig. 1, showing the lymphocytic composition of the graft. Note that the cells are almost all large, medium, and small lymphocytes with uniform nuclear structure. Few erythroblasts and myeloid cells are to be seen. The structure and composition of these adult spleen grafts, which were capable of reconstituting the neonatally thymectomized mice, is very similar to that of the spleens of normal mice; × 1000 (oil immersion).



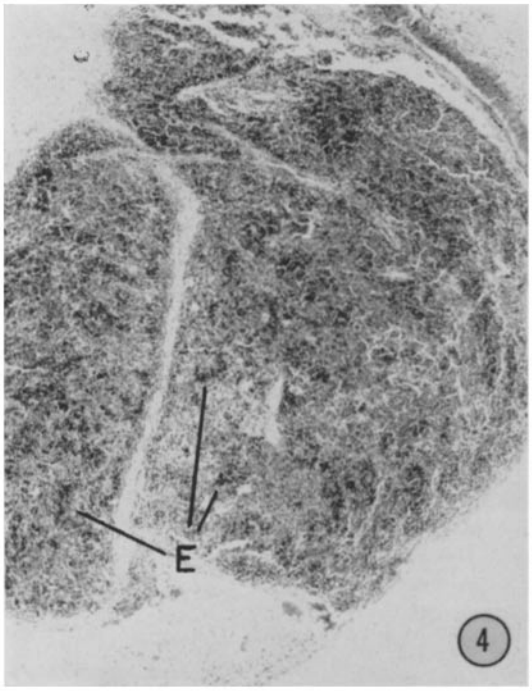
(Yunis *et al.*: Immunologic reconstitution of thymectomized mice)

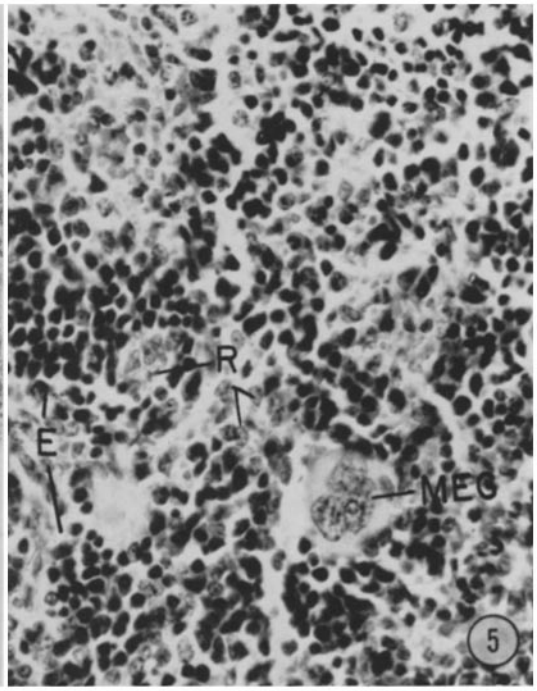
PLATE 51

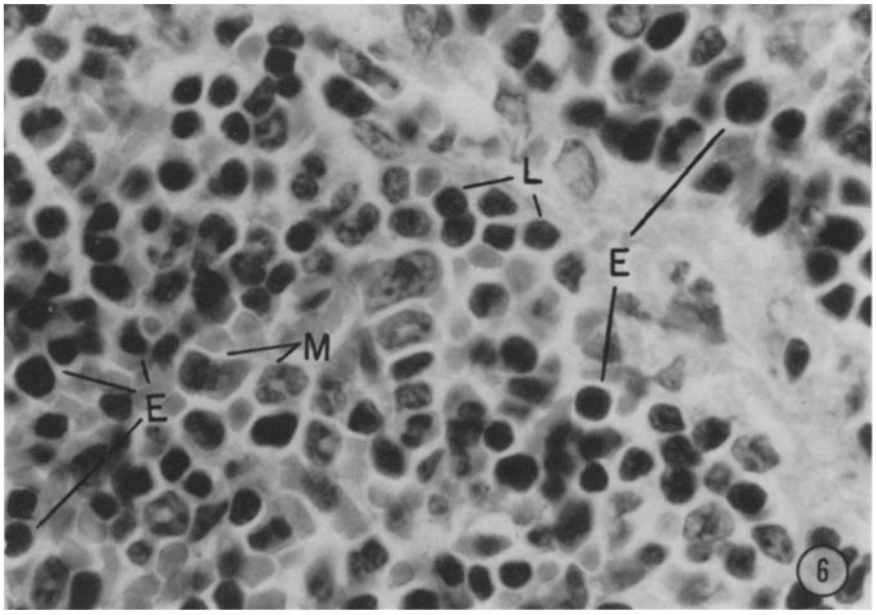
FIG. 4. Low power view of a newborn C_3H spleen, grafted in a neonatally thymectomized C_3H recipient at 3 days of age, and recovered at 6 weeks. It will be seen that the graft did not develop lymphoid cells or follicular structure, but was composed primarily of stromal-reticular tissue and frequent clusters of erythroid elements (*E*) and areas of myelopoiesis. Such grafts did not restore neonatally thymectomized recipients physiologically or immunologically. × 40.

FIG. 5. Medium power view of the neonatal spleen graft shown in Fig. 4. Note the clusters of erythroid-normoblastic cells (*E*), open areas composed primarily of reticulum cells (*R*), and megakaryocytes (*MEG*). × 400.

FIG. 6. High power view of a portion of Fig. 4. Note the erythroid (*E*) and myeloid (*M*) elements and the very few lymphocytes (*L*). The striking differences in cellular composition of the spleen grafts shown here and in Fig. 3 are similar to the differences observed between spleens of newborn mice and mice 2 to 3 months of age; × 1000 (oil immersion).







(Yunis *et al.*: Immunologic reconstitution of thymectomized mice)

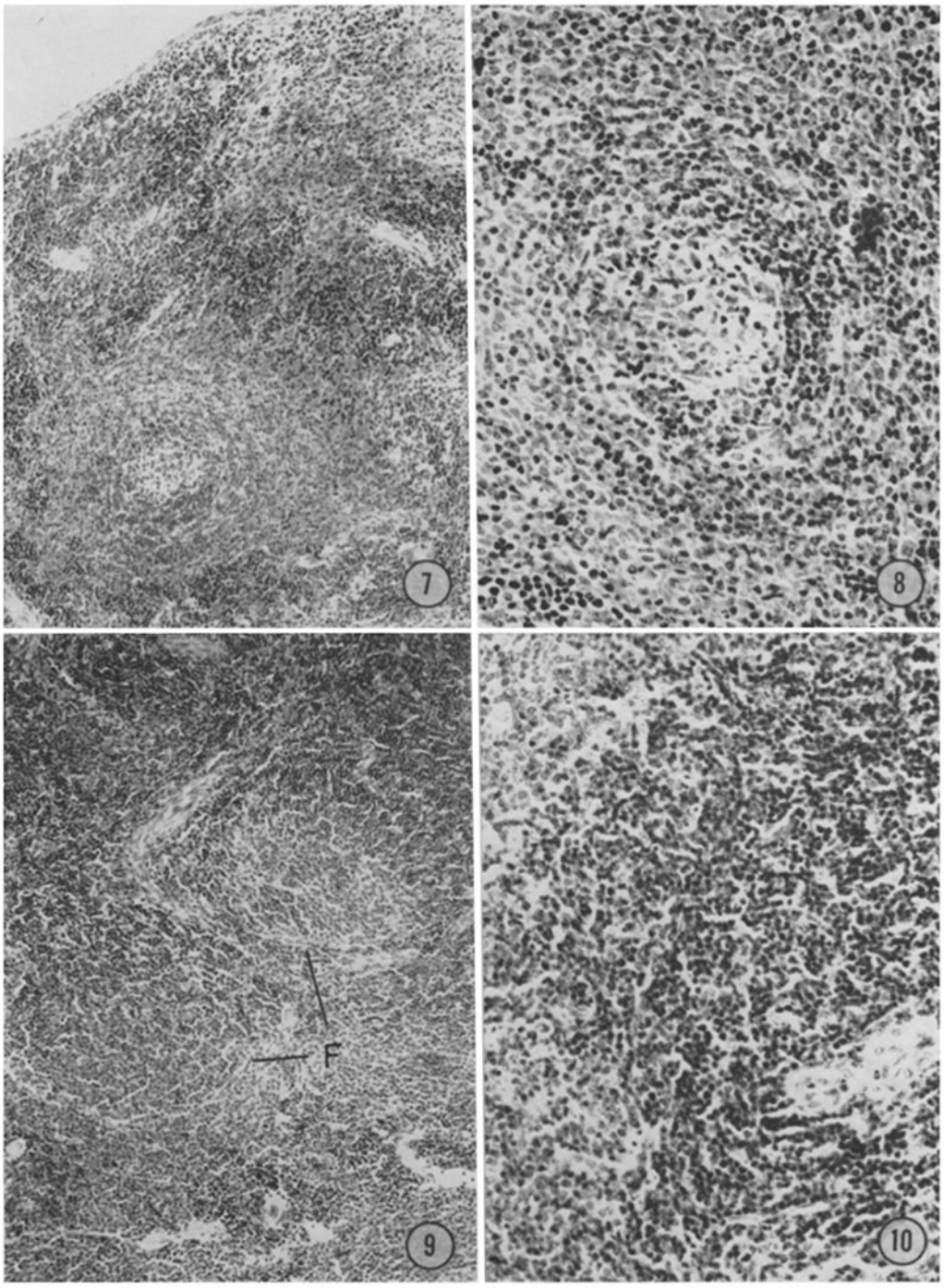
PLATE 52

FIG. 7. Spleen from a neonatally thymectomized C_3H mouse at 6 weeks of age. Although some lymphocytes are present, their number is so small that the spleen is lacking in characteristic organization and follicular arrangement. × 100.

FIG. 8. Higher power view of the follicular region of a depleted neonatally thymectomized mouse spleen. The sparsity of lymphocytes and the abundance of reticulum cells are evident. Follicular centers are to be found but they contain few lymphocytes. × 400.

FIG. 9. The spleen of a 3-month-old C_3H mouse thymectomized in the neonatal period and reconstituted by the intraperitoneal injection of 100 million $(A \times C_3H)F_1$ "hemiallogeneic" spleen cells. Note the abundance of lymphocytes and the well developed follicular organization (*F*). × 100.

FIG. 10. Higher power view of a splenic follicle in the spleen illustrated in Fig. 9. Note the abundance of small lymphocytes in this region. Reticulum stroma is, of course, present as in Fig. 8, but the greater concentration of lymphocytes tends to obscure this component. × 400.



(Yunis *et al.*: Immunologic reconstitution of thymectomized mice)

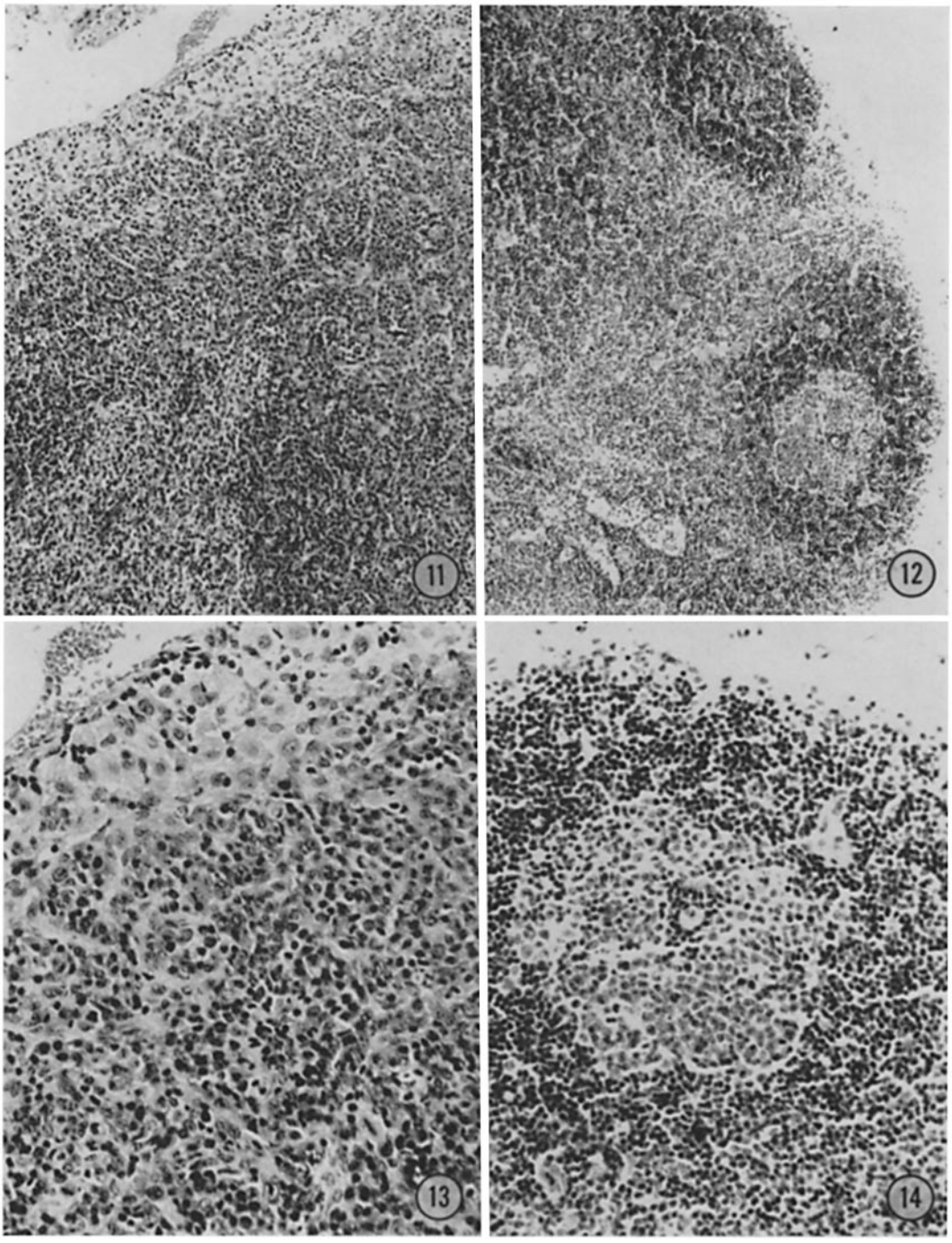
PLATE 53

FIG. 11. Low power view of an axillary lymph node of a 6-week-old C_3H mouse that had been thymectomized at birth. The lack of follicle development and the relative sparsity of small lymphocytes are apparent. × 40.

FIG. 12. Low power view of the axillary lymph node of a 120-day-old neonatally thymectomized C_3H mouse whose wasting disease was reversed by injection of 200 million CE spleen cells (weak histocompatibility barrier). The follicular structure and lymphocyte numbers approach those of normal lymph nodes. Note the thick collars of small lymphocytes surrounding the well developed follicles in the cortex of this node. × 40.

FIG. 13. Higher power view of the lymph node illustrated in Fig. 11. Note the relative deficiency of small lymphocytes and the relative abundance of larger lymphoid cells and reticulum cells. × 250.

FIG. 14. Higher power view of the lymph node illustrated in Fig. 12. Note the well developed collar of small lymphocytes in the neonatally thymectomized mouse whose wasting disease was reversed by intraperitoneal injection of 200 million allogeneic spleen cells. × 250.



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