CARCINOGEN-INDUCED TUMORS OF THE THYMUS

IV. Humoral Influences of Normal Thymus and Functional Thymomas and Influence of Postthymectomy Period on Restoration*

By OSIAS STUTMAN, M.D., EDMOND J. YUNIS, M.D., and ROBERT A. GOOD, M.D.

(*From the Departments of Laboratory Medicine, Pediatrics, and Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455*)

(Received for publication 7 May 1969)

Studies with functional nonlymphoid thymomas showed that when the tumors were grafted into neonatally thymectomized allogeneic hosts, immunological restoration was mediated by the host's own lymphoid cells (1). Comparable results had been previously reported with thymus grafts (2–6), while spleen cells produced adoptive restoration mediated by donor cells (4, 7). It was suggested that the tumoral nonlymphoid stroma of the thymus may act in an indirect way inducing differentiation and/or expansion of a population of potentially competent cells in the lymphoid tissues of thymectomized hosts (1). This hypothesis was supported by evidence of the functional capacity of the thymomas when enclosed in cell-impenetrable diffusion chambers (8).

In a preliminary report (9), we observed a decrease of the restorative capacity of a functional thymoma in neonatally thymectomized conventional mice when the treatment was delayed after thymectomy. Treatment became ineffective when performed at 50 days of age or later. These results suggested that a population of cells in the tissues of the thymectomized hosts, capable of responding to the inductive or expanding action of the thymoma, decreased progressively with time after neonatal thymectomy in the absence of thymic function. Our present results also indicate that with delay of the treatment a similar decrease in restoring capacity was observed when thymus grafts, thymus in diffusion chambers, and thymomas alone or in diffusion chambers were used.

Materials and Methods

Animals.—Inbred mice of the A, C3Hf/Bi, DBA/2, and C57BL/1 strains were used. Strain details and animal care were described in a previous paper (1).

Technical Procedures.—The techniques for neonatal thymectomy, thymoma and thymus

* Aided by Public Health Service research grant CA-10445 from the National Cancer Institute; by grant AI 00798 from the National Institute of Allergy and Infectious Disease; by the National Foundation—March of Dimes; and by the Minnesota Heart Association and the University of Minnesota Graduate School.

grafting, skin grafting, cell suspension preparation, and delayed hypersensitivity reactions to sheep red cells have been described in previous publications (1, 8).

Diffusion chambers were prepared with lucite rings and Millipore diffusion filters of 0.22 μ average pore size (Millipore Filter Corp., Bedford, Mass.). Details and descriptions of the chambers have been reported previously (8).

Graft-versus-host assays were performed using 10×10^6 spleen cells from the C3Hf/Bi donors injected intraperitoneally into 8-day old (C3HxC57BL/1)F_1 hybrids. Spleen indices were obtained 8 days later as described in previous publications (1, 7).

Nonlymphoid Functional Thymomas.—Two nonlymphoid functional thymomas of A and C3H/Bi origin were used. The tumors were found 200 days after intrathymic application at birth of 0.1 mg of 7, 12-dimethylbenzanthracene. When used in the present experiments, the thymoma A was in its fifth transplant generation and the thymoma C3H/Bi was in its third generation. Description of the morphological and functional characteristics of these thymomas have been reported previously (1, 8). The A sarcoma of the A strain used as control in some experiments (Table VII and VIII) was a nonfunctional mediastinal sarcoma obtained 200 days after intramediastinal injection at birth of 0.1 mg of 7, 12-dimethylbenzanthracene; it was in its fourth transplant generation. Thymus graft donors were always 20-day old male and female mice.

Restoration Criteria.—We considered neonatally thymectomized animals to have been restored by the different treatments if they fulfilled the following criteria (for restoration): (*a*) complete thymectomy at the termination of the experiment (microscopic analysis of the mediastinal contents); (*b*) 200 day survival; (*c*) capacity to reject DBA/2 skin grafts in less than 15 days when tested at 90–100 days of age; (*d*) capacity to produce delayed hypersensitivity to sheep red cells when tested at 150–160 days of age; and (*e*) capacity of their spleen cells to initiate graft-versus-host reactions when injected into adequate F_1 hybrids. The presence of all five criteria were essential for considering a particular animal restored.

Experimental Designs.—

Effect of age at grafting: Neonatally thymectomized A or C3Hf/Bi mice were grafted with thymus or thymomas at different time intervals after thymectomy, ranging from 5 to 50 days (see Tables I–IV). Restoration was indicated by the five criteria mentioned earlier. The following experiments were performed: (*a*) neonatally thymectomized A mice grafted subcutaneously at different ages with A thymus or A thymoma (Table I); (*b*) neonatally thymectomized C3Hf/Bi mice grafted subcutaneously at different ages with C3Hf/Bi, (C3HxA)F_1 or A thymus, or with A thymoma (Table II); (*c*) neonatally thymectomized C3Hf/Bi mice grafted intraperitoneally at different ages with C3Hf/Bi or A thymus, or with A or C3H/Bi thymomas enclosed within cell-impermeable diffusion chambers (Table IV).

Attempts to reverse the posthymectomy-wasting syndrome: As in previous publications (5, 7), wasting was defined by a decrease of at least 10% body weight or by a failure to gain weight for at least 10 days after reaching a certain level. Treatments were performed 1 day after clear-cut evidence of wasting syndrome was obtained in conventional neonatally thymectomized C3Hf/Bi or A mice. Wasting appeared at approximately 60–85 days of age. Restoration criteria were similar to those previously described, except that delayed hypersensitivity tests were not done in the present group of experiments. The following experiments were performed: (*a*) wasted C3Hf/Bi or A mice grafted with one or five subcutaneous or intraperitoneal thymus grafts of C3Hf/Bi or A origin, or with subcutaneous strain A thymoma grafts (Table V); (*b*) consisted of the same model except that thymuses or thymomas of A or C3H/Bi origin were implanted intraperitoneally within cell-impermeable diffusion chambers (Table VI); (*c*) wasted A mice treated with (i) subcutaneous grafts of A thymoma or A sarcoma, (ii) intraperitoneal injection of 200×10^6 spleen cells from 5-day old or 45-day old neonatally thymectomized A mice, and (iii) combination of subcutaneous tumor grafts with intraperitoneal spleen cells

(Table VII); (iv) wasted C3Hf/Bi mice treated with: subcutaneous grafts of A thymoma or A sarcoma; intraperitoneal injection of 200 or 400 $\times$ 10^6 spleen cells from 5-day old or 45-day old neonatally thymectomized C3Hf/Bi mice; and a combination of subcutaneous tumors and intraperitoneal spleen cells (Table VIII).

RESULTS

Effect of Age at Treatment.—Table I shows the influence of delaying treatment after neonatal thymectomy in strain A mice on the restorative capacity of syngeneic functional thymoma and thymus grafted subcutaneously. A progressive

TABLE I

Effect of Age at Treatment on Restoration of Neonatally Thymectomized Strain A Mice with Syngeneic Thymoma or Thymus Subcutaneous Grafts

Age at grafting*	Number restored per number treated‡	
	A thymoma	A thymus
days	%	%
5	16/20 (80)	8/10 (80)
10	9/10 (90)	8/10 (80)
15	16/24 (66)	9/12 (75)
20	11/17 (64)	7/8 (87)
30	8/20 (40)	8/13 (61)
40	7/30 (25)	7/12 (58)
45	6/32 (18)	5/12 (41)
50	2/15 (13)	5/16 (31)

* Subcutaneous grafting of 1 $\times$ 10^6 thymoma cells or one thymus from 20-day old donors

‡ For restoration criteria see text.

decrease in effectiveness was observed when treatments were delayed. When grafting was performed between 5 and 20 days after neonatal thymectomy, restoration was observed in 72% (52/71) mice treated with thymoma and 80% (32/40) mice treated with thymus. By contrast, when grafting was performed between 30 and 50 days after thymectomy, restoration was observed in 23% (23/97) mice treated with thymoma and 47% (25/53) mice grafted with thymus. Comparable results are shown in Table II, when neonatally thymectomized C3Hf/Bi mice were grafted subcutaneously with syngeneic, hemiallogeneic, and allogeneic thymus grafts, or with the functional strain A thymoma. Restoration was observed in 75% (70/93) treated with syngeneic thymus at 5–20 days of age and 46% (37/80) treated with syngeneic thymus at 30–50 days of age. When the animals were grafted with (C3HxA)F_1 thymus, 62% (25/40) mice were restored when grafted at 5–20 days and 26% (15/56) were restored when grafted at 30–50 days of age. Allogeneic A strain thymus was less effective and only 24% (12/46) animals were restored when grafted at 5–20 days of age and 9% (6/66) when treated at 30–50 days of age. Restoration with the func-

tional strain A thymoma was 65% (82/126) for the groups grafted at 5–20 days and 22% (32/148) for the groups grafted at 30–50 days of age. These results indicate that a progressive decrease on the restorative capacity of syngeneic or

TABLE II

Effect of Age at Treatment on Restoration of Neonatally Thymectomized C3Hf/Bi Mice with Allogeneic Thymoma or Syngeneic or Allogeneic Thymus Subcutaneous Grafts

Age at grafting*	Number restored per number treated‡			
	C3Hf/Bi thymus	(C3HxA)F1 thymus	A thymus	A thymoma
days	%	%	%	%
5	16/20 (80)	ND§	3/10 (30)	22/30 (73)
10	16/20 (80)	9/12 (75)	3/12 (25)	16/20 (80)
15	18/24 (75)	8/14 (57)	3/12 (25)	22/40 (55)
20	12/16 (75)	8/14 (57)	3/12 (25)	10/16 (62)
25	8/13 (61)	ND	ND	12/20 (60)
30	16/26 (61)	6/12 (50)	3/16 (18)	11/45 (24)
35	8/14 (57)	ND	2/14 (14)	8/20 (40)
40	6/12 (50)	5/14 (35)	1/12 (8)	7/30 (23)
45	4/12 (33)	3/14 (20)	0/12	5/35 (14)
50	3/16 (18)	1/16 (6)	0/12	1/18 (5)

* Subcutaneous grafting of 1×10^6 thymoma cells or one thymus from 20-day old donors.
‡ For restoration criteria see text.
§ ND, not done.

TABLE III

Effect of Age at Treatment on Restoration of Neonatally Thymectomized C3Hf/Bi Mice with Intraperitoneal Thymus Grafts

Age at treatment*	Number restored per number treated‡		
	C3Hf/Bi thymus	(C3HxA)F1 thymus	A thymus
days	%	%	%
10	8/10 (80)	8/10 (80)	3/10 (30)
20	8/10 (80)	8/10 (80)	3/10 (30)
30	10/12 (83)	9/13 (69)	3/12 (25)
40	6/10 (60)	5/10 (50)	0/10
50	3/10 (30)	3/10 (30)	0/10

* Intraperitoneal grafting of one thymus from 20-day old donors.
‡ For restoration criteria see text.

allogeneic thymus or thymoma grafts takes place when the treatment is delayed after neonatal thymectomy.

When syngeneic or allogeneic thymus grafts were implanted intraperitoneally in neonatally thymectomized C3Hf/Bi mice, comparable decrease in effectiveness was observed (Table III).

When syngeneic or allogeneic thymus grafts or functional thymomas were

grafted intraperitoneally within cell-impenetrable diffusion chambers in neonatally thymectomized C3Hf/Bi mice, a marked decrease in effectivity was observed when the treatment was performed after 25 days of age (Table IV). When treatment was performed at 10–20 days, restoration was observed in 40% (17/42) mice treated with syngeneic C3Hf/Bi thymus, 39% (17/43) treated with allogeneic A thymus, 65% (39/60) grafted with syngeneic C3H/Bi thymoma, and 56% (24/43) animals grafted with allogeneic strain A thymoma. When the

TABLE IV

Effect of Age at Treatment on Restoration of Neonatally Thymectomized C3Hf/Bi Mice with Intraperitoneal Diffusion Chambers Containing Thymus or Thymoma Grafts

Age at grafting*	Number restored per number treated‡				
	C3Hf/Bi thymus	A thymus	C3Hf/Bi thymoma	A thymoma	Empty chamber
days	%	%	%	%	
10	7/14 (50)	7/13 (53)	13/20 (65)	8/12 (66)	0/10
15	7/15 (46)	7/15 (46)	13/65 (65)	8/15 (53)	0/12
20	3/13 (23)	3/15 (20)	13/20 (65)	8/16 (50)	0/10
25	2/12 (16)	1/12 (8)	6/22 (27)	2/10 (20)	0/10
30	1/12 (8)	0/20	3/14 (21)	6/22 (27)	0/15
35	1/12 (8)	0/12	0/12	3/14 (21)	0/10
40	0/12	1/19 (5)	0/13	3/14 (21)	0/10
45	0/12	0/12	0/15	1/17 (5)	0/5
50	0/12	0/15	0/13	0/12	0/5

* Intraperitoneal implantation of diffusion chambers (lucite rings with 0.22 μ average pore size filters) containing 2 × 2 mm fragments of thymomas or thymus from 20-day old donors.

‡ For restoration criteria see text.

TABLE V

Reversal of Postthymectomy Wasting in Neonatally Thymectomized C3Hf/Bi and A Mice with Thymus or Thymoma Grafts

Treatment*	Route	Restoration‡	
		C3Hf/Bi	A
		%	%
1 thymus C3Hf/Bi	s.c.	0/42	0/14
1 thymus A	s.c.	0/11	0/28
5 thymuses C3Hf/Bi	s.c.	13/60 (21)	4/40 (10)
5 thymuses A	s.c.	4/40 (10)	5/26 (19)
Thymoma A	s.c.	0/49	0/48
1 thymus C3Hf/Bi	i.p.	2/32	0/10
1 thymus A	i.p.	0/12	1/22 (4)
5 thymuses C3Hf/Bi	i.p.	19/29 (65)	4/41 (9)
5 thymuses A	i.p.	4/21 (19)	3/29 (10)

* Grafting performed subcutaneously or intraperitoneally after onset of wasting.

‡ Number restored per number treated. For restoration criteria see text.

treatment was performed at 25–50 days of age, restoration decreased to 5% (4/72) for the syngeneic thymus group, 2% (2/90) for the allogeneic thymus group, 10% (9/89) for the syngeneic thymoma group, and 16% (15/89) for the allogeneic thymoma group. The decrease in effectiveness of the treatments, when delayed after neonatal thymectomy is more accentuated when thymus or thymomas are implanted within cell-impenetrable diffusion chambers.

Attempts to Reverse Postthymectomy Wasting.—Neonatally thymectomized C3Hf/Bi and A mice were treated after the onset of the postthymectomy-wasting syndrome, generally at 55–75 days of age. As reported previously (5),

TABLE VI

Attempts to Reverse Postthymectomy Wasting in Neonatally Thymectomized C3Hf/Bi and A Mice with Thymus or Thymomas in Diffusion Chambers

Treatment*	Restoration‡	
	C3Hf/Bi	A
Empty diffusion chamber	0/10	0/11
1 thymus C3Hf/Bi	0/19	0/13
1 thymus A	0/12	0/13
5 thymuses C3Hf/Bi	0/43	0/29
5 thymuses A	0/20	0/21
Thymoma C3H/Bi	0/37	0/10
Thymoma A	0/30	0/33

* Intraperitoneal implantation of diffusion chambers (lucite rings and 0.22 μ Millipore filters) after onset of wasting. Thymus donors were 20-day old normal females.

‡ Number restored per number treated. For restoration criteria see text.

Table V shows that when grafted subcutaneously or intraperitoneally, five thymuses of syngeneic origin and to less degree of allogeneic strains can reverse the wasting syndrome after its onset. One thymus graft is usually ineffective. Thymoma grafts in syngeneic or allogeneic host mice with wasting disease were ineffective (Table V). Table VI shows that one thymus, multiple thymuses or, functional thymomas of syngeneic or allogeneic origin enclosed in cell-impenetrable diffusion chambers are incapable of reversing the postthymectomy wasting after its onset. All animals died 5–45 days after the treatment was performed and showed no restoration of immunological responses.

Effect of Spleen Cells from Thymectomized Donors.—Table VII shows that wasted A mice could be restored only with intraperitoneal spleen cells from 5-day old neonatally thymectomized syngeneic donors in combination with subcutaneous grafts of the strain A functional thymoma. Spleen cells from 45-day old neonatally thymectomized donors were ineffective. Table VIII shows similar experiments performed in wasted C3Hf/Bi mice. As in the previous experiments, spleen cells from 5-day old neonatally thymectomized syngeneic donors injected intraperitoneally were effective in reversing the wasting when associated with

subcutaneous grafts of the functional strain A thymoma. Spleen cells from 45-day old neonatally thymectomized donors in association with thymoma grafts were ineffective. In both types of experiments thymoma grafts alone, spleen cells, or spleen cells in association with a nonfunctional strain A sarcoma were

TABLE VII

Attempts to Reverse Postthymectomy Wasting in Neonatally Thymectomized A Mice with Strain A Thymoma and Spleen Cells from Neonatally Thymectomized Strain A Mice

Treatment performed after onset of wasting, approximately at 40–60 days of age.

Tumor graft*	Spleen cells‡	Age of spleen cell donors	Number restored per number treated
	$\times 10^6$	*days*	%
A thymoma	None	—	0/16
A sarcoma	None	—	0/13
None	None	—	0/12
None	200	5	0/12
None	200	45	0/12
A thymoma	200	5	4/14 (28)
A thymoma	200	45	0/24
A sarcoma	200	5	0/11

* Subcutaneous injection of 1×10^6 tumor cells.

‡ Intraperitoneal injection of spleen cells from neonatally thymectomized strain A mice of 5 or 45 days of age.

TABLE VIII

Attempts to Reverse Postthymectomy Wasting in Neonatally Thymectomized C3Hf/Bi Mice with Strain A Thymoma and Spleen Cells from Neonatally Thymectomized C3Hf/Bi Mice

Treatment performed after onset of wasting, approximately at 40–60 days.

Tumor graft*	Spleen cells‡	Age of cell donor	Number restored per number treated
	$\times 10^6$	*days*	%
A thymoma	None	—	0/25
A sarcoma	None	—	0/12
None	None	—	0/30
None	200	5	0/13
None	400	5	0/29
None	400	45	0/21
A sarcoma	200	5	0/13
A sarcoma	400	45	0/11
A thymoma	200	5	6/20 (30)
A thymoma	400	5	6/19 (31)
A thymoma	200	45	0/19
A thymoma	400	45	1/21 (5)

* Subcutaneous injection of 1×10^6 tumor cells.

‡ Intraperitoneal injection of spleen cells from neonatally thymectomized C3Hf/Bi mice of 5 or 45 days of age.

ineffective in reversing the wasting syndrome after its onset. Further, they were unable to induce restoration of any of the parameters studied.

DISCUSSION

Restoration of neonatally thymectomized mice with thymus grafts suggested that the thymus is acting in an indirect way, since all the immunocompetent cells are derived from the host (2–6). Comparable results were obtained with functional thymomas (1), or thymus, or thymomas within cell-impenetrable diffusion chambers.[1] The cell-impenetrable chamber experiments suggested that the thymus may act by means of a humoral diffusible factor (10). Other mechanisms of thymic function include a traffic through thymic stroma of cells of hemopoietic origin (11) and a seeding out of a thymus-derived cell population to the peripheral lymphoid tissue (12). The three possible mechanisms of thymic action are not mutually exclusive, although the quantitative contribution of each component under physiological conditions is unknown. It would seem from our present results that two factors are necessary for normal development and continued function of the thymus: an intact reticuloepithelial framework, related to the inductive function; and a supply of "stem" cells, probably of hemopoietic origin, sensitive to the inductive action of the thymus.

Our present results can be summarized as follows: (*a*) there is a progressive decrease of restorative effectiveness of thymoma or thymus grafts when the treatment of neonatally thymectomized mice is delayed; (*b*) early restoration between 5 and 20 days after thymectomy is effective, while the number of restored animals is markedly decreased following attempts to achieve restoration between 30 and 50 days postthymectomy; (*c*) comparable results are obtained with subcutaneous grafts, intraperitoneal grafts, or grafts within cell-impenetrable diffusion chambers (Tables I–IV). It is possible that the population of cells capable of responding to the inductive action of the thymoma or thymus in diffusion chambers may represent a population that received "thymic influence" before thymectomy was performed, and that the humoral function of the thymus can expand solely such population of "postthymic" cells in the peripheral tissues of the host (13). In mice reared in conventional environment, the decay of the postthymic cells with time may be partly explained by an exhaustion due to lack of replacement in absence of thymic function and/or through commitment to immunological functions.

A decrease in effectiveness, although less marked, was observed when syngeneic thymus grafts were used (Table I–III), indicating that the difference may be due to the thymic stroma itself. It can be theorized that the population of "postthymic" cells arises through a traffic of bone marrow cells to the thymus, and that only normal syngeneic thymus (as opposed to allogeneic thymus,

[1] Stutman, O., E. J. Yunis, and R. A. Good. Unpublished observations.

thymomas, or thymus in diffusion chambers) can provide optimal conditions for traffic of cells of hemopoietic origin and for the building up of a population of "postthymic" cells capable of response to the inductive or expanding humoral activity of the thymus (13). The comparison between spleen cells from 5-day old and 45-day old neonatally thymectomized mice for their capacity to reverse the postthymectomy-wasting syndrome when given in association with thymoma grafts also indicates that a population of cells sensitive to the function of the thymoma is present in the spleen cells of 5-day old thymectomized mice and is absent in the spleen cells from 45-day old neonatally thymectomized hosts (Tables VII and VIII). All these results are compatible with the view that a special cell is present in the peripheral tissues of the neonatally thymtecomized host. This special cell is sensitive to the inductive or expanding action of the thymus. This intermediate cell requires the presence of the thymus for renewal of its population and will decrease progressively in absence of thymic function. Additional experiments (13) indicate that this type of cell is already present in lymphohemopoietic tissues of newborn mice but is absent in the hemopoietic tissues of embryonic mice. The difference between the "embryonic" and "newborn" type of cell is that the latter is sensitive to the humoral action of the thymus (thymoma or thymus in diffusion chambers), while the former needs the normal thymic stroma to achieve the state of immunological competence. This suggests that thymus traffic must occur for this influence to be exercised (13).

The effect of allogeneic thymus grafts (Tables II and III) is comparable to previous experiments, suggesting that differences in influence between allogeneic and syngeneic or F_1 hybrid parent combinations may be attributed to a graft-versus-host reaction (4–6, 14).

The experiments on reversal of the postthymectomy-wasting syndrome deserve comment. Wasting in thymectomized mice can be prevented or delayed under germfree conditions, suggesting that environmental factors influence the pathogenesis of this syndrome (15). However, the wasting model is still of importance since it mimics dramatically the syndrome observed in the human lymphopenic states (16, 17). In previous publications we showed that the postthymectomy syndrome could be easily reversed adoptively with immunocompetent cells, and that multiple thymus grafts could also effectively reverse the syndrome and restore such animals to health and immunologic competence (4, 5, 7). Thymus grafts showed that restoration was eventually mediated by the host's own lymphoid cells (5). The present results confirm previous finding (5, 18), showing that the only effective treatment with thymus grafts of the postthymectomy wasting after its onset was the implantation of multiple thymus grafts (Table V). On the other hand, Table VI shows that when only the humoral activity of the thymus was employed (multiple thymuses or thymoma in diffusion chambers), no reversal of the wasting syndrome was observed. These results suggest that an initial "cellular" stage is needed for the reversal of the

syndrome. This cellular stage could be represented by a component of thymus-derived cells (12) and/or by traffic of host cells through the thymus grafts (11). The quantitative difference between multiple and single thymus grafts may be due to the acuteness of the disease and the marked decrease of "postthymic" cells with time after thymectomy. This difference is indicated in experiments reported here. One article in which postthymectomy wasting was reversed with multiple thymus grafts within diffusion chambers in 35% of the treated animals has been presented (19). Timing of the treatment, strain variations, environmental factors, restoration criteria, and techniques used for construction of the chambers (0.30 as opposed to 0.22 μ mean pore size filters) could easily account for the difference in results. The two last points may be important since (*a*) our restoration criteria, besides survival, include the return to normal of two immunological parameters in the same animal; and (*b*) our report states that the 0.30 μ mean pore size permits some cell traffic across the filters (8).

SUMMARY

A progressive decrease of the restoring effectivity of syngeneic or allogeneic thymus and functional thymoma grafts was observed when the treatment of neonatally thymectomized mice was delayed. Early treatment (5–20 days postthymectomy) was effective, while the number of restored animals was markedly decreased after late treatment (30–50 days postthymectomy). Similar results were obtained with subcutaneous or intraperitoneal thymus grafts and with thymus grafts within cell-impenetrable diffusion chambers. After the onset of the postthymectomy-wasting syndrome the only successful treatment was the implantation of multiple thymus grafts. On the other hand, single thymus grafts, thymoma grafts, or thymus or thymoma within diffusion chambers were ineffective. When spleen cells from 5-day old or 45-day old neonatally thymectomized animals were given in association with thymoma grafts, only the cells derived from the 5-day old thymectomized mice proved effective in restoring wasted thymectomized hosts. These results suggest that a population of cells sensitive to the action of the thymus decreases progressively with time in the absence of thymic function.

The authors wish to thank Mary Jo O'Connor, June M. Smith, and Antoinette Hanson for their able assistance.

BIBLIOGRAPHY

1. Stutman, O., E. J. Yunis, and R. A. Good. 1968. Carcinogen-induced tumors of the thymus. I. Restoration of neonatally thymectomized mice with a functional thymoma. *J. Nat. Cancer. Inst.* **41:**1431.
2. Dalmasso, A. P., C. Martinez, K. Sjodin, and R. A. Good. 1963. Studies on the role of the thymus in immunobiology. Reconstitution of immunologic capacity in mice thymectomized at birth. *J. Exp. Med.* **118:**1089.

3. Miller, J. F. A. P. 1964. Effect of thymic ablation and replacement. *In* The Thymus in Immunobiology. R. A. Good and A. E. Gabrielsen, editors. Hoeber-Harper. New York. 436.
4. Yunis, E. J., H. R. Hilgard, C. Martinez, and R. A. Good. 1965. Studies on immunologic reconstitution of thymectomized mice. *J. Exp. Med.* **121**:607.
5. Stutman, O., E. J. Yunis, C. Martinez, and R. A. Good, 1967. Reversal of post-thymectomy wasting disease in mice by multiple thymus grafts. *J. Immunol.* **98**:79.
6. Stutman, O., E. J. Yunis, and R. A. Good. 1969. Tolerance induction with thymus graft in neonatally thymectomized mice. *J. Immunol.* **103**:92.
7. Stutman, O., E. J. Yunis, and R. A. Good. 1969. Reversal of post-thymectomy wasting in mice with immuno-competent cells: Influence of histocompatibility differences. *J. Immunol.* **102**:87.
8. Stutman, O., E. J. Yunis, and R. A. Good. 1969. Carcinogen-induced tumors of the thymus. III. Restoration of neonatally thymectomzed mice with thymomas in cell impermeable chambers. *J. Nat. Cancer Inst.* **43**:499.
9. Stutman, O., E. J. Yunis, and R. A. Good. 1967. Effect of age at grafting on protection of thymectomized mice with thymic tumor. *Proc. Amer. Ass. Cancer. Res.* **8**:65.
10. Osoba, D. 1965. The effects of thymus and other lymphoid organs enclosed in Millipore diffusion chambers on neonatally thymectomized mice. *J. Exp. Med.* **122**:633.
11. Ford, C. E. 1966. Traffic of lymphoid cells in the body. *In* Ciba Foundation Symposium, The Thymus: Experimental and Clinical Studies. G. E. W. Wolstenholme and R. Porter, editors. Little, Brown and Co. Boston. 131.
12. Davies, A. J. S., E. Leuchars, V. Wallis, and P. C. Kollers. 1966. The mitotic response of thymus derived cells to antigenic stimulus. *Transplantation.* **4**:438.
13. Stutman, O., E. J. Yunis, and R. A. Good. 1969. Thymus: An essential factor in lymphoid repopulation. *Transplant Proc.* **1**:614.
14. Stutman, O., E. J. Yunis, P. O. Teague, and R. A. Good. 1968. Graft-versus-host reactions induced by transplantation of parental strain thymus in neonatally thymectomized F_1 hybrid mice. *Transplantation.* **6**:514.
15. McIntire, K. R., S. Sell, and J. F. A. P. Miller. 1964. Pathogenesis of the post-neonatal thymectomy wasting syndrome. *Nature (London).* **204**:151.
16. Hoyer, J. R., M. D. Cooper, M. A. Gabrielsen, and R. A. Good. 1968. Lymphopenic forms of congenital immunologic deficiency diseases. *Medicine.* **47**:201.
17. Bergsma, D., and R. A. Good, editors. 1968. Birth defects. *In* Immunologic Deficiency Diseases in Man. The National Foundation, New York. **4**:(1).
18. Schaller, R. T., J. Schaller, and J. K. Stevenson. 1967. Reversal of wasting syndrome in thymectomized mice by multiple syngeneic or allogeneic thymus grafts. *J. Nat. Cancer. Inst.* **38**:287.
19. Schaller, R. T., and J. K. Stevenson. 1967. Reversal of post-thymectomy wasting syndrome with multiple thymus grafts in diffusion chambers. *Proc. Soc. Exp. Biol. Med.* **124**:199.