THE ROLE OF THE THYMUS IN DEVELOPMENT OF IMMUNOLOGIC CAPACITY IN RABBITS AND MICE*

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Although the thymus gland has been known from antiquity, its role in the body economy has remained enigmatic until recently. During the past year, however, a flurry of reports have given the first indications of the role of this organ in developmental biology. In mammals the thymus develops from the third and fourth pharyngeal pouches in early embryonic life, reaches maximal relative size near the time of birth, and then undergoes a gradual involution. In man the thymus appears in the 10 mm embryo, reaches maximal relative size in the neonate, attains maximal absolute size in the 12-year-old child, and then gradually decreases in relative and absolute size as maturity is reached (1).

Our interest in the thymus developed in 1953 when we (2, 3) discovered that a patient with "acquired agammaglobulinemia" had developed a marked immunologic deficiency and an abnormality of the thymus—a benign thymoma—at about the same time. Removal of the thymoma, which was primarily an epithelial stromal overgrowth of the thymus pathologically, failed to alter either the protein abnormality or the immunologic defect. Since that time seven cases of the combined occurrence of these two disorders have been reported (4–8), and in no instance has removal of the thymic tumor restored immunologic function or the protein deficit. Nonetheless, it seems clear that the association of these two rare conditions has been far too frequent to be explained by chance alone.

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In an attempt to exploit the initial clinical observation, we (9, 10) studied the effect of thymectomy on the immunologic capacity of adult rabbits. In these experiments, which confirmed the earlier findings of Harris *et al.* (11) we could demonstrate no effect of thymectomy on immunologic capacity. These observations seemed consonant with earlier findings (12–14) that the thymus, unlike other lymphoid organs, remains aloof from antigenic stimulation in adult animals and does not seem to form antibody, except perhaps when antigen is injected directly into its substance (15).

When it became apparent that the bursa of Fabricius (16, 17), a thymus-like organ of the chicken, plays an important role in the early development of immunologic potential in that animal, we turned to study of the effect of thymectomy in newly born animals on the subsequent development of immunologic capacity. Archer and Pierce, from this laboratory, presented the first results of these studies at the meeting of the American Society of Immunologists in 1961 (18). In substance, we found that thymectomy in newborn rabbits had an effect on development of immunologic potential. Fichtelius *et al.* (19), in work with guinea pigs, and more recently Miller (20) and Martinez *et al.* (21), in independent studies of inbred strains of mice, made similar observations.

It is the purpose of this report to present our findings linking the thymus to the development of immunologic capacity in the mouse and the rabbit. It will be shown that in both of these species, but most consistently and most completely in the mouse, the thymus plays an essential role in maturation of immunologic function and in the development of the anatomic integrity of the lymphoid tissues. Further, evidence will be presented indicating that thymectomy in mice as late as 35 to 40 days after birth may affect capacity to reject skin homotransplants and may also affect resistance of F_1 hybrid mice to the development of immunologic runt disease (homologous disease) (22, 23).

Materials and Methods

Rabbits:

New Zealand albino rabbits were obtained from a single local breeder in the terminal stages of gestation, allowed to deliver in secluded cages in our animal quarters, and the young were divided into litter mate groups. The young were kept with the dam until self-sufficient, and then separated and fed Purina rabbit chow, greens, and water *ad libitum*.

Thymectomy in Rabbits.—Rabbits were anesthetized with ether and thymectomized before the 7th day of life through a median incision of the chest wall. The thymus was removed with a glass aspirating pipette or by surgical dissection, and the incision closed with silk suture.

Antigenic Stimulation and Antibody Production in Rabbit.—In two experiments, antigenic stimulation was provided with bovine serum albumin (BSA) in a dosage of 50 mg per kg, given intravenously when the rabbits were 5 to 8 weeks of age. Anti-BSA content of the serum was measured qualitatively by the method of Swift *et al.* (24) on the 14-day serum samples, and quantitatively by the ammonium sulfate precipitation method of Farr (25) on 14- and 21-day serum samples. In a third group of animals, antigenic stimulation was provided by 8×10^9 particles per kg of T_2 coliphage, given intravenously at 14 to 18 weeks of age. Phage neutralization procedures (26) were carried out on 7-day serum samples. All blood was obtained by cardiac puncture.

Transplantation Immunity in Rabbits.—Skin homografting was performed essentially by the method described by Stetson and Demopoulos (27). Each grafted rabbit received 2 autografts, 1 on each ear, and 2 homografts, 1 from another New Zealand rabbit and the other from a Dutch rabbit.

Mice:

Mice of the following strains were used in these studies: $C_{57}Bl/1$, C_3H, Ce, DBA/2, A, Balb/C, $(A \times C_3H)F_1$, $(A \times C_{57}Bl)F_1$, $(Balb/C \times DBA/2)F_1$, $(C_3H \times DBA/2)F_1$, and $(Balb/C \times A)F_1$. The C_3H mice were obtained from the stock originally maintained by Dr. J. J. Bittner. Newborn mice remained with the mother until old enough to eat by themselves, and were then fed Purina fox chow and water *ad libitum*.

Thymectomy in Mice.—Mice were thymectomized by a modification of the technique used by Gross (28). This modification is essentially the same as that reported by Dischler and Rudali (29).[1]

Antigenic Stimulation and Antibody Production in Mice.—In mice the antigenic stimulation was provided by intraperitoneal injection of 2×10^{10} particles of bacteriophage T_2 at 2 months of age. The animals were bled 7 days later from the retro-orbital venous plexus into capillary tubes. The tubes were centrifuged at 4°C at 1500 *G*, and the serum removed and tested for virus neutralization at 37°C. Serum samples were diluted 1:50 in 20 per cent normal rabbit serum in 0.9 per cent NaCl, and incubated with 10^6 particles of bacteriophage per ml in the reaction tubes. The bacteriophage particles remaining active after 2 and 24 hours of incubation were assayed in the usual way (26). The mice were sacrificed and autopsied 2 weeks after bleeding.

Transplantation Immunity in Mice.—Skin homotransplantation immunity was tested by placing 1.5 by 2 cm skin homografts on the back of the tested animals. The grafting technique has been described (30).[2] Tumor transplantation immunity was studied by taking a mammary adenocarcinoma which originated spontaneously in an A breeder female, and transplanting pieces of this tumor into the subcutaneous tissue of C_3H mice. "Takes" were recorded when the tumor in the foreign host had reached 1 cm in size and was growing.

Growth of Mice.—The mice were weighed at frequent intervals on a Mettler balance, and the weights of thymectomized and sham-operated animals compared at 4, 8, and 12 weeks.

Immunologic Activity of Spleen and Lymph Node Cells in Mice.—Mice of A, Balb/C, and C_3H strains were thymectomized or sham-operated during the 1st or 6th day of life and sacrificed 2 months later. Their spleens or lymph nodes were made into suspensions in Locke-Ringer's saline solution. Each dose of 0.1 cc contained 10 million viable spleen cells. The hybrid recipients, which were 8 days old at the time of transfer, were sacrificed 8 days later, and the Simonsen assay of graft *versus* host reaction was determined (31).

Runt Disease in Mice.—$(A \times C_3H)F_1$ and $(A \times C_{57}Bl)F_1$ mice were thymectomized or sham-operated at birth or at 40 days of age. Those thymectomized in the newborn period were given A strain spleen cells intraperitoneally at 35 days of age; those thymectomized at 40 days of

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[1] The immediate surgical mortality of mice and rabbits in the early experiments was often quite high, in some experiments as high as 90 per cent. More recently, the immediate mortality of the thymectomy procedure in newborn mice in our laboratories has been in the neighborhood of 10 to 20 per cent.

[2] The criteria of skin homograft acceptance varied with the conditions of the individual study, and details are furnished in the text and/or table in the Results section. When mice thymectomized at birth were grafted, however, the observation period always continued to clear-cut slough of the graft or the death of the animal. These animals are short-lived, and no mouse is included in the tabulations as a graft survival unless it lived for at least 35 days after grafting.

age were given adult A strain spleen cells intravenously or intraperitoneally 10 days after the surgery.

Histologic Studies.—Spleen, lymph node, thymus, and gut tissue were taken at birth and at intervals after birth from thymectomized and control mice, placed in either 10 per cent neutral formalin or 100 per cent ethyl alcohol, sectioned, and stained with hematoxylin and eosin, or methyl green–pyronin. The methyl green–pyronin staining technique used in our laboratory has been previously described (32).

TABLE I

*Effect of Thymectomy in Rabbits Less Than 5 Days of Age on Production of Antibody to Bovine Serum Albumin**

Group	14 day bleeding			21 day bleeding		
	Number of rabbits	Antibody‡ gamma nitrogen/100 ml	Significance of difference	Number of rabbits	Antibody‡ gamma nitrogen/100 ml	Significance of difference
Thymectomized	7	Mean = 6.6 Range = 0 to 19.5	$P < 0.01$	7	Mean = 9.8 Range = 0 to 42.5	$P < 0.01$
Unthymectomized (sham-operated and non-operated)	10	Mean = 52.4 Range = 0 to 129.0		10	Mean = 62.2 Range 14.5 to 207.9	

* Antibody was measured by the ammonium sulfate precipitation technique of Farr (25).

‡ Results are expressed as gamma nitrogen of I^{131}-labeled BSA bound per 100 ml of undiluted serum determined at that dilution of serum which bound 33 per cent of 0.01 gamma of BSA nitrogen added.

RESULTS

Effect of Neonatal Thymectomy on Development of Immunologic Capacity in Rabbits.—

In the first experiment, rabbits under 5 days of age were either thymectomized, sham-operated, or left alone. Challenge with BSA (50 mg/kg) was carried out when the animals were 7 to 8 weeks old. In the second experiment, thymectomy or sham operation was performed when the rabbits were 5 to 7 days of age, and BSA administered intravenously at 5 to 7 weeks. In the third experiment, the rabbits were thymectomized or sham-operated at 0 to 3 days of age, and immunized with 8×10^9 particles of T_2 coliphage per kg at 14 to 18 weeks of age.

In the first experiment, none of 7 thymectomized rabbits produced sufficient antibody to BSA to be detected by the qualitative capillary tube precipitin technique, whereas 8 of 10 sham-operated or unoperated litter mate controls formed demonstrable precipitating antibody. One rabbit whose thymus had been only partially removed produced sufficient antibody to be detected by this precipitation technique. Quantitative data on the same serums, obtained by

the ammonium sulfate precipitation method of Farr (25) are summarized in Table I. The thymectomized rabbits had significantly smaller quantities of antibody at 14 and 21 days than did sham-operated or unoperated controls.

Table II records the quantitative data from the second experiment, involving thymectomy at 5 to 7 days after birth and challenge with BSA at 5 to 7 weeks. Antibody production was reduced both 14 and 21 days after antigen administration in the thymectomized group. Although these results suggested immunologic feebleness of thymectomized rabbits, the differences were not significant statistically. The capillary tube precipitin method revealed antibody in 14-day serum samples of 4 of 9 thymectomized rabbits and 16 of 27 unthymectomized animals.

TABLE II

*Effect of Thymectomy in Rabbits 5 to 7 Days of Age on Production of Antibody to Bovine Serum Albumin**

Group	14 day bleeding		21 day bleeding	
	Number of rabbits	Antibody‡ gamma nitrogen/100 ml	Number of rabbits	Antibody‡ gamma nitrogen/100 ml
Thymectomized	9	Mean = 23.3 Range = 0 to 57.0	8	Mean = 25.1 Range = 7.8 to 58.4
Unthymectomized (sham-operated and non-operated)	26	Mean = 31.0 Range = 0 to 95.7 The differences are not statistically significant.	21	Mean = 41.8 Range = 0 to 178.0

* Antibody was measured by the ammonium sulfate precipitation technique of Farr (25).

‡ Results are expressed as gamma nitrogen of I^{131}-labeled BSA bound per 100 ml of undiluted serum determined at that dilution of serum which bound 33 per cent of 0.01 gamma of BSA nitrogen added.

In the third experiment, the rabbits were thymectomized or sham-operated before 3 days of age and challenged with T_2 coliphage 14 to 18 weeks later. The results of bacteriophage neutralization by 7-day serum samples are recorded in Table III. In this experiment the thymectomized animals again formed less antibody than did the control animals, but the results were not statistically different.

In a final study, rabbits which had been thymectomized or sham-operated at birth were tested for abnormalities in homotransplantation immunity.

Four rabbits completely thymectomized before 3 days of age and 3 sham-operated controls were subjected to skin homotransplantation 6 months later. In essence, 1 cm circular autografts were exchanged between the 2 ears of each rabbit. In addition, the right ear received a 1 cm circular homograft from a Dutch strain donor and the left ear a 1 cm circular homograft from a homologous donor of New Zealand stock.

The results, summarized in Table IV, indicate that thymectomy in the neonatal period does not interfere significantly with the capacity of rabbits to reject skin homotransplants.

These experiments indicate that rabbits thymectomized shortly after birth form less antibody after they have matured than do sham-operated and un-

TABLE III

*Antibody Production to T_2 Coli Bacteriophage in Rabbits Thymectomized at 0 to 3 Days of Age**

Group	Per cent surviving 1 hr.‡	Per cent surviving 2 hrs.‡
Thymectomized at 0 to 3 days of age	86	85
	66	63
	75	67
	96	71
	78	56
	76	67
	83	64
	69	41
Mean ± standard error	78 ± 5	64 ± 5§
Sham-operated or unoperated controls	66	45
	56	31
	80	34
	83	72
	97	89
	26	5
	— (Plating error)	72
	54	23
Mean ± standard error	66 ± 11	46 ± 10§
Diluent control	Not determined	95 ± 0.5

* Rabbits were immunized with 8×10^9 particles of phage at 14 to 18 weeks of age.

‡ Figures refer to the per cent of surviving phage following addition of 10^5 particles/ml to tubes containing 5 cc of a 1:100 dilution of antiserum.

§ The difference between the means of the 2-hour determinations is not statistically significant (P value between 0.05 and 0.1).

operated controls. The difference between the thymectomized and control groups was least pronounced in the second experiment (Table II), the study which involved the latest thymectomy (at 5 to 7 days of age), suggesting that the state of maturation of the lymphoid tissue of the rabbit in the immediate neonatal period may be important. In both other experiments, the animals were thymectomized before 5 days of age. In one the difference between the thymec-

tomized and control animals was statistically significant; in the other it approached significance.

The final experiment indicates that thymectomy carried out immediately after birth in the rabbit does not reduce immunologic capacity sufficiently to permit skin homotransplantation. It seems likely that more consistent and more complete inhibition of the development of the immunologic capacity of the rabbit would require even earlier thymectomy, probably considerably before birth (33).

The rabbit, then, although revealing immunologic incompetence following early thymectomy in some experiments, is not the experimental animal of

TABLE IV

Effect of Neonatal Thymectomy on Skin Homotransplantation in Rabbits

Group	Rabbit No.	Dutch rabbit skin homotransplant		New Zealand rabbit skin homotransplant	
		Day hemorrhagic	Day sloughed	Day hemorrhagic	Day sloughed
Thymectomized at 0 to 3 days of age*	1	—	18	9	18
	2	‡	‡	8	16
	3	10	14	10	14
	4	9	17	9	17
Sham-operated at 0 to 3 days of age	5	10	18	10	18
	6	10	14	10	14
	7	—	18	9	18

* These animals had previously been shown to be quantitatively deficient with respect to antibody production against T_2 bacteriophage.

‡ Technical failure of graft.

choice for studies of the role of the thymus in development of immunologic capacity at this time.

Immunologic Incompetence of Thymectomized Mice.—

In these experiments newborn mice of the DBA/2 strain were divided into two groups. Members of one group were thymectomized, while members of the second group were sham-operated as controls. Two months later, each mouse was injected intraperitoneally with 2×10^{10} particles of bacteriophage T_2. The phage neutralization procedures were performed on 7-day serum samples.

Recorded on Table V are the results of this study which reveal a definite difference in neutralizing capacity of the antiserums from thymectomized and sham-operated animals. After the 24 hour incubation, neutralization of bacteriophage was complete in the serums of all the sham-operated mice, whereas only one of the serums from mice subjected to thymectomy showed complete neu-

tralization. By contrast, the serums from thymectomized animals were in most instances no more effective in neutralizing bacteriophage than was the normal rabbit serum diluent. However, 3 of the mice subjected to thymic extirpation at birth appeared to form antibody. In 1 of these the antibody production approached that of the control mice; in the other 2 it was reduced but still significant. These data are rendered more meaningful when the results of autopsies

TABLE V

*Effect of Neonatal Thymectomy on Capacity of Mice to Form Antibody Against T_2 Bacteriophage**

Group	Mouse No.	Per cent phage remaining in serum after 2 hrs.' incubation‡		Per cent phage remaining in serum after 24 hrs.' incubation‡	
Completely thymectomized at 0 to 24 hrs.	1	96		38	
	2	85		37	
	3	96	Mean = 89	40	Mean = 37
	4	75		40	
	5	92		30	
Sham-operated at 0 to 24 hrs.	1	20		0	
	2	30		0	
	3	35		0	
	4	15	Mean = 40	0	Mean = 0
	5	40		0	
	6	90		0	
	7	50		0	
Incompletely thymectomized at 0 to 24 hrs.	1§	50		0	
	2‖	77	Mean = 67	10	Mean = 7
	3‖	75		12	
20 per cent normal rabbit serum control		88		40	

* Antigenic stimulus was provided, at 60 days of age, by 2×10^{10} bacteriophage particles. Antibody was assayed by phage neutralization procedures on serums from bleeding 7 days following antigen administration.

‡ Antisera were diluted 1:50 in a 20 per cent normal rabbit serum-saline diluent.

§ ⅓ thymus present at autopsy.

‖ Traces of thymus present at autopsy.

on these animals are considered. At autopsy all the sham-operated mice were found to have an intact and normal thymus. Animals thymectomized at birth fell into two groups: 5 showing complete absence of thymic tissue, and 3 showing some residual thymic tissue. One of these 3 had a thymus approximately ⅓ normal size and the 2 others had small traces of thymus. Of real significance is the observation that the thymectomized mice which at autopsy showed no residual thymus formed no demonstrable antibody. The "thymectomized"

mouse having a residual thymus 1/3 the normal size formed antibodies about as well as did sham-operated controls, and the 2 mice with smaller residuals of thymus formed amounts of virus-neutralizing antibodies intermediate between the 2. The results of antibody production in these three groups may be compared on Table V.

Acceptance of Homografts across Minor Histocompatibility Barriers in Mice.— In our initial experiments concerning the effect of neonatal thymectomy on homotransplantation immunity, inbred strains of mice similar to one another at the H-2 histocompatibility locus but differing at other genetic loci were employed. Implicit in the design of these experiments was the concept that if thymectomy in the neonatal period should produce a quantitative reduction in capacity to develop transplantation immunity, it might be revealed by testing homografts between strains of animals differing from one another in weaker histocompatibility antigens (34). To this end, four inbred strains of mice were studied. These included the DBA/2, C_3H, and Ce strains, and F_1 hybrids resulting from the cross between Balb/C and DBA/2 parents.

In an initial experiment DBA/2 mice were divided into two groups. Members of one group were thymectomized at 0 to 24 hours of age, and members of another group were thymectomized at 30 days of age. Sham-operated controls were prepared for each age group. At 35 days of age, all mice were given skin homotransplants taken from (Balb/C x DBA/2)F_1 hybrid donors. In these experiments, donors and recipients were of like sex and similar age, and the skin homografts were applied according to the method generally in use in this laboratory (30). The grafts were inspected at least 3 times per week for a period of at least 6 months or until they had definitely sloughed.

The results are recorded in Table VI where it will be seen that thymectomy performed on DBA/2 strain mice in the immediate neonatal period frequently prolonged survival of (Balb/C x DBA/2)F_1 hybrid skin grafts. By contrast, thymectomy performed in the DBA/2 mice at 30 days of age had no significant effect on survival of (Balb/C x DBA/2)F_1 skin homografts.

In additional studies, C_3H mice thymectomized at 30 days of age were grafted with skin from Ce mice 5 days later. The results are summarized in Table VI. It can be seen in the table that although homografts between members of these two strains are regularly rejected in the untreated mouse, C_3H mice thymectomized at 30 days of age subsequently accepted skin grafts from Ce strain donors and retained them indefinitely. These observations, already published in preliminary form (21) established, as did those of Miller (20), that development of homotransplantation immunity is profoundly affected by extirpation of the thymus at birth in mice. Evidence was also obtained from these studies that in certain strains of mice, but not in others, extirpation of the thymus as late as 30 days of age also has some effect on immunologic capacity.

Acceptance of Tumor Homografts across the H-2 Histocompatibility Locus.— Since it was considered likely that relatively minor manipulations of immuno-

logic potential might permit homotransplantation between strains of mice which differ very slightly, it was deemed necessary to study the effects of neonatal thymectomy on transplantation immunity which is dependent on genetic differences at the H-2 histocompatibility locus which controls the strongest histocompatibility antigens in mice (34). To test the possibility of inhibiting homotransplantation immunity between strains of mice differing at the H-2 histocompatibility locus, mice of the C_3H and A strains were employed.

TABLE VI

Effect of Thymectomy on Survival of Skin Homografts across Relatively Weak Histocompatibility Barriers

Group	Source of skin graft*	No. of mice with prolonged graft survival‡	No. of grafts accepted permanently
		No. of mice grafted	No. of mice grafted
DBA/2 mice thymectomized at 0 to 24 hrs.	(Balb/C x DBA/2)F_1	27/27	17/27
DBA/2 mice sham-operated at 0 to 24 hrs.	(Balb/C x DBA/2)F_1	0/20	0/20
DBA/2 mice thymectomized at 30 days	(Balb/C x DBA/2)F_1	9/21	0/21
DBA/2 mice sham-operated at 30 days	(Balb/C x DBA/2)F_1	0/10	0/10
C_3H mice thymectomized at 30 days	Ce	19/19	12/19
C_3H mice sham-operated at 30 days	Ce	0/10	0/10

* All animals were grafted at 35 days of age.

‡ Graft survival was considered prolonged if it was longer than the longest of the control homografts. When (Balb/C x DBA/2)F_1 skin was grafted on control DBA/2 mice, the grafts survived an average of 18 days, with a range from 14 to 28 days. When Ce skin was grafted on control C_3H mice, the grafts survived an average of 21 days, with a range from 14 days to 5 weeks.

C_3H mice were thymectomized, sham-operated, or splenectomized during the first 24 hours of life. One to 2, 22, and 50 days following surgery, mice of each group were grafted in the subcutaneous tissue with mammary adenocarcinoma spontaneously arising in an A strain breeder female. Incidences of "takes" and progressive growth of the tumors to destruction and death of the recipients were recorded.

The results of these experiments are summarized in Table VII where it will be seen that thymectomy in the neonatal period facilitated transplantation of mammary adenocarcinoma across the H-2 histocompatibility barrier. The age of the animal at the time of the tumor implant was a significant variable, however; as shown in Table VII, 13 of 21 (62 per cent) of sham-operated control animals accepted grafts in the neonatal period, while all 11 thymectomized

animals did so, a difference which is statistically significant ($P < 0.01$). The high rate of tumor acceptance in the thymectomized group was more evident when grafting was done at 22 or 50 days of age, since rejection of the transplant was the rule in control sham-operated and splenectomized mice grafted at those ages. The high rate of acceptance of grafts by control animals in the newborn period apparently reflected their immunologic immaturity.

In a second set of experiments, groups of 35-day-old C_3H mice were prepared by thymectomy, splenectomy, thymectomy-splenectomy, or sham-operation. Twelve and 55 days after surgery, mice of all groups received subcutaneous grafts of A strain mammary adenocarcinoma.

TABLE VII

Homotransplantation of Mammary Adenocarcinoma Across the H-2 Histocompatability Barrier in Thymectomized Mice

Group	Survival of A strain tumor homografts*				
	Transplanted 1 to 2 days PO‡	Transplanted 12 days PO	Transplanted 22 days PO	Transplanted 50 days PO	Transplanted 55 days PO
C_3H mice thymectomized at 0 to 24 hrs.	11/11		13/15	4/7	
C_3H mice sham-operated at 0 to 24 hrs.	13/21		1/20	0/10	
C_3H mice splenectomized at 0 to 24 hrs.	—		1/13	0/10	
C_3H mice thymectomized at 35 days		0/10			0/13
C_3H mice sham-operated at 35 days		0/28			0/13
C_3H mice splenectomized at 35 days		0/12			0/13
C_3H mice thymectomized and splenectomized at 35 days		0/12			0/10

* Each entry shows the number of tumor "takes" (recorded when the tumor had reached 1 cm in size and was continuing to grow) over the number of animals receiving the transplants.

‡ PO indicates postoperatively.

In no instance did this tumor take and grow to destruction of the host. These results are summarized in Table VII.

Homotransplantation of Skin across the H-2 Histocompatibility Barrier in Mice.—In our original experiments (21) it was shown that thymectomy at birth regularly permitted transplantation across weak histocompatibility barriers, but ordinarily only prolonged survival of the grafts when the two strains of mice differed according to histocompatibility genes (H-2) controlling strong histocompatibility antigens. Autopsy studies in the earlier experiments, however, often revealed minute amounts of residual thymus tissue in these animals. With perfection of the technique of neonatal thymectomy in our laboratories, it was possible to remove all of the thymus in the newborn mouse, and studies were once again carried out using strains of mice which differed at the H-2 genetic locus.

In this set of experiments, complete thymectomy was performed in newborn mice of the C_3H and DBA/2 strains. Litter mates were prepared by sham operation, subtotal thymectomy, and splenectomy. Additional C_3H mice were thymectomized, or both splenectomized and thymectomized, at 35 days of age. The C_3H mice completely thymectomized, sham-operated, incompletely thymectomized, or splenectomized at birth were grafted with skin from adult (A x C_3H)F_1 donors when they reached 40 days of age. The DBA/2 mice prepared at birth were grafted with skin from adult C_3H donors at 40 days of age, and the C_3H mice thymectomized, or splenectomized and thymectomized, at 35 days of age were grafted with skin from (A x C_3H)F_1 donors either 1 week or 2 months following operation.

The results summarized in Table VIII indicate that whereas mice sham-operated, splenectomized, or incompletely thymectomized at birth had no survival of skin homografts across this strong histocompatibility barrier, those

TABLE VIII

Effect of Thymectomy on the Capacity of Mice to Reject Skin Homografts across the H-2 Histocompatibility Barrier

Recipient strain	Skin donor strain	Age at grafting	Thymectomy 0 to 24 hrs.	Subtotal* thymectomy 0 to 24 hrs.	Sham operation 0 to 24 hrs.	Splenectomy 0 to 24 hrs.	Thymectomy 35 days	Splenectomy-thymectomy 35 days
		days						
DBA/2	C_3H	40	9/12‡	0/9	0/19	—	—	—
C_3H	(A x C_3H)F_1	40	12/14	0/10	0/16	0/8	—	—
		42	—	—	—	—	0/8	0/21
		95	—	—	—	—	0/6	0/9

* 90 to 95 per cent of thymus removed.

‡ Each table entry shows the number of grafts accepted over the number of mice grafted and surviving at least 35 days after grafting.

in which thymectomy was complete at birth regularly accepted skin homografts even across these strong antigenic barriers.

Mice thymectomized, or subjected to both splenectomy and thymectomy, at 35 days of age, showed no increased incidence of survival of skin homografts across these strong histocompatibility barriers, whether grafted 1 week or 2 months following the operation.

Transplantation of Male Skin Isografts on Female Mice Thymectomized in the Neonatal Period.—It has been shown by Eichwald and Silmser (35) that in certain inbred strains of mice the females will regularly reject male skin isografts, whereas male → male, female → male, and female → female skin isografts are always successful. This phenomenon, known as the Eichwald-Silmser phenomenon, has been interpreted as being an immunologic process dependent on a genetic difference between male and female conditioned by the Y chromosome of the males of these strains of mice. Immunologic tolerance produced by

intravenous injection of spleen cells into the females from the males either at birth (36, 37) or in larger doses later in life (38, 39) argues strongly for the operation of immunologic processes in the rejection of male skin by females in these strains of mice.

In Table IX are presented data which indicate that thymectomy performed immediately after birth renders female $C_{57}Bl/1$ mice capable of accepting isografts of male skin from mice of the same strain. By contrast, $C_{57}Bl$ females either thymectomized, or both thymectomized and splenectomized, at 35 days of age, do not take skin isografts when the skin is transplanted 1 week following the extirpation of these organs.

Failure of Spleen and Lymph Node Cells from Thymectomized Mice to Induce Graft versus Host Reactions.—In an effort to study further the immunologic capacity of mice thymectomized at birth, an analysis was made of immunologic

TABLE IX

*Effect of Thymectomy on Survival of Skin Isografts from Male to Female $C_{57}Bl/1$ Mice**

Groups of mice studied			
Thymectomy at birth‡	Sham-operation at birth‡	Thymectomy at 35 days§	Thymectomy and splenectomy at 35 days§
6/6	1/15	1/12	0/7

* Each entry records the number of mice accepting grafts over the number grafted, excluding any animal that survived for less than 35 days after grafting. The observation period was the remaining lifetime of the animals thymectomized at birth, and 4 months in the other groups.

‡ Skin grafted at 40 days of age.

§ Skin grafted one week after operation.

activity of their spleen and lymph node cells, using the graft *versus* host assay of histocompatibility of Simonsen (31).

Mice of the A, Balb/C, and C_3H strains, and F_1 hybrids resulting from the cross between A and $C_{57}Bl$, Balb/C and A, and C_3H and DBA/2 strains were used. Animals of the A, Balb/C and C_3H strains were either thymectomized or sham-operated during the 1st day of life, with the exception of a series of C_3H mice operated on at 6 days of age. Two months after the surgery, the animals were sacrificed, and suspensions of their spleen or lymph node cells were prepared for immediate injection into appropriate 8-day-old F_1 hybrid recipients. A control group of the same F_1 strain was injected with isologous spleen or lymph node cells at the same time. The Simonsen assay, an evaluation of splenic enlargement produced in the F_1 hybrid recipients by the graft *versus* host reaction, was performed when the recipients were sacrificed 8 days later.

Table X reveals that the spleen and lymph node cells of 60-day-old mice thymectomized at birth were immunologically inactive and thus incapable of

producing a graft *versus* host reaction upon intraperitoneal injection into F_1 hybrid recipients. By contrast, spleen and lymph node cells from mice sham-operated at the same time, regularly induced development of splenic enlargement and other manifestations of homologous disease in the appropriate F_1 recipients. It will also be seen in the table that thymectomy carried out in C_3H mice even as late as 6 days after birth had a marked effect on the capacity of the spleen and lymph node cells of these animals to produce a graft *versus* host reaction in hybrid hosts.

TABLE X

Immunologic Incompetence of Spleen and Lymph Node Cells from Thymectomized Mice

Donor strain*	Prior treatment of donor	F_1 hybrid recipient‡	Spleen index§	
			Spleen cells	Lymph node cells
A	Thymectomy at 1 to 24 hrs.	(A x C_{57}Bl)F_1	1.04	—
A	Sham-operated at 1 to 24 hrs.	(A x C_{57}Bl)F_1	2.35	—
Balb/C	Thymectomy at 1 to 24 hrs.	(Balb/C x A)F_1	1.03	1.22
Balb/C	Sham-operated at 1 to 24 hrs.	(Balb/C x A)F_1	2.09	3.68
C_3H	Thymectomy at 1 to 24 hrs.	(C_3H x DBA/2)F_1	0.97	1.05
C_3H	Thymectomy at 6 days	(C_3H x DBA/2)F_1	1.08	1.12
C_3H	Sham-operated at 1 to 24 hrs.	(C_3H x DBA/2)F_1	2.21	3.04

* The spleen and lymph node cell donors were sacrificed at approximately 60 days of age.

‡ Each mouse was injected intraperitoneally with 10 million donor cells at 8 days of age, and weighed and killed 8 days later.

§ The spleen weight for each animal was converted to a relative spleen weight (mg spleen weight/100 gm body weight). A mean was figured for each experimental group and each control group (F_1 hybrid animals of the appropriate strain injected with isologous spleen or lymph node cells) and the spleen index determined by dividing the mean of the experimental group by the mean for the appropriate control group. The size of the experimental groups varied from 8 to 18 animals in the spleen cell series, and 4 to 7 in the lymph node series.

These data indicate, then, that the peripheral lymphoid cells (spleen and lymph node cells) of mice thymectomized shortly after birth are immunologically defective. The immunologic inactivity of these peripheral lymphoid cells from mice thymectomized at birth was apparent even when the recipients had perfectly normal thymuses of their own, and by all criteria of growth, development, and general well-being, were capable of providing a normal environment for cell function. These observations argue strongly against the operation of essential non-specific processes which in thymectomized mice might be defective and capable of limiting the immunologic function of spleen or other lymphoid cells.

Growth Failure and Early Death of Mice Thymectomized in the Neonatal Period.—Failure of immunologic mechanisms was not the only deficit observed in mice thymectomized in the immediate neonatal period. One of the most striking characteristics of these animals was failure of both linear growth and weight gain. In Table XI are summarized data from a typical experiment which

TABLE XI

*Effect of Neonatal Thymectomy on Growth and Survival of C_3H Mice**

Group	Weight in gm at ages indicated		
	4 wks.	8 wks.	12 wks.
Control sham-operated	12.5	22.6	27.0
	13.6	25.0	29.2
	13.2	23.9	25.8
	13.4	22.1	26.2
	12.8	24.2	26.0
	13.7	19.2	23.4
	12.4	18.3	20.8
	13.5	18.1	20.9
	13.1	19.2	22.4
	11.0	19.7	23.8
Mean	12.9 ± 0.3‡	21.2 ± 0.8	24.5 ± 0.8
Thymectomized at 0 to 24 hrs. of age.	7.8	Dead	Dead
	8.3	15.6	13.6
	8.8	11.9	Dead
	12.4	20.8	15.6
	8.0	14.9	12.7
	7.7	12.0	Dead
	8.2	Dead	Dead
	9.3	13.1	13.4
Mean	8.8 ± 0.5	14.7 ± 1.4	13.8 ± 0.6

* Mice were thymectomized at 0 to 24 hours of age.

‡ Mean ± standard error of the mean.

illustrates the regular failure of weight gain in mice completely thymectomized in the neonatal period. Fig. 1 illustrates the linear growth failure in these thymectomized animals. In this figure, 2 mice thymectomized at birth are compared to a litter mate that was sham-operated at the same time but left with an intact thymus.

In addition, the thymectomized mice were short-lived, generally dying between the 50th and 90th day of life. The cause of death, although often due to infection, was not always discernible on gross postmortem examination.

Production of Runt Disease in Thymectomized F_1 Hybrid Mice Injected with Parental Strain Lymphoid Cells.—Because of the runting observed in thymectomized mice otherwise untreated, and because of some of the similarities in appearance between mice thymectomized at birth and those runted as a consequence of a homologous graft *versus* host reaction, it was of interest to determine whether mice thymectomized at birth are more susceptible to the production of

TABLE XII

Occurrence of Homologous Disease in Thymectomized Mice Following Injection of Parent Strain Spleen Cells

Recipient group	Operation and time performed	No. of A strain cells injected	No. of deaths / No. of animals	Time of death (days following injection)*
(A x C_3H)F_1	Thymectomy at 0 to 24 hours	200 million‡	6/6	16 ± 1.6
	Sham-operation at 0 to 24 hours	200 million‡	2/10	46, 28
	Thymectomy at 0 to 24 hours	None	7/7	30 ± 2.6§
(A x C_3H)F_1	Thymectomy at 40 days	200 million‖	0/10	—
	Sham-operation at 40 days	200 million‖	0/9	—
	Thymectomy at 40 days	None	0/12	—
(A x $C_{57}Bl$)F_1	Thymectomy at 40 days	150 million¶	9/9	23 ± 2.4
	Sham-operation at 40 days	150 million¶	8/14	32 ± 1.7
	Thymectomy at 40 days	None	0/11	—

* Mean ± standard error of the mean.

‡ Spleen cells from 6 month old donors injected intraperitoneally 35 days following operation.

§ Days from 35 days of age to death.

‖ Spleen cells from 6 month old donors injected intravenously 10 days following operation.

¶ Spleen cells from 10 to 12 month old donors injected intraperitoneally 10 days following operation.

homologous disease than are sham-operated mice whose thymuses remain intact. The results summarized in Table XII indicate that both the development of immunologic runt disease and the severity of the process were favored by neonatal thymectomy. The (A x C_3H)F_1 animals thymectomized at birth and injected with parent A strain cells at 35 days of age developed runt disease more readily than did sham-operated controls, and the animals dying of the homologous disease died considerably sooner in the thymectomized group studied.

Thymectomy at 40 days of age affected susceptibility of the (A x $C_{57}Bl$)F_1

hybrids to runt disease, but had no discernible effect on the incidence and severity of homologous disease in (A x C_3H)F_1 animals.

These studies indicate that thymectomy at birth, and in some strain combinations as late as 40 days of age, renders the F_1 hybrid more susceptible to the damaging effect of the graft *versus* host reaction than are sham-operated control animals. Thymectomy carried out at 40 days of age did not interfere with the growth or apparent well-being of the F_1 hybrid animals studied in this experiment.

Morphology of the Lymphoid Tissue of Thymectomized Mice.—In the mouse at birth, the thymus, an organ of endodermal-epithelial origin, is the only organ with true lymphocytes. Figs. 2, 4, and 5 illustrate the thymic structure at birth. By contrast, the spleen, lymph nodes, and gut are either myelopoietic or simple reticular in nature, and contain very few, if any, true lymphoid cells. Figs. 3, 6, and 7 illustrate this in the spleen. Structural changes are discernible during the 1st week of life, and are progressive during the next month, as the lymphoid organs increasingly show adult characteristics: typical follicular structure and well-organized, fully developed cortical-medullary architecture in the lymph nodes, and the red and white pulp characteristic of the normal spleen. Illustrated on Figs. 8 and 9 is the spleen of a 40-day-old mouse sham-operated at birth. In striking contrast are the lymphoid organs of the mouse thymectomized at birth. The lymph node and spleen are often relatively small and lack the structure and lymphoid cells observed in the mature control animals. The spleen of a 40-day-old mouse thymectomized at birth is shown in Figs. 10 and 11 for comparison with the sham-operated control.

DISCUSSION

The data presented in this report indicate that in both rabbits and mice a deficiency in capacity to form circulating antibodies is produced when the animals have been completely thymectomized early enough in the neonatal period. The effect of neonatal thymectomy is somewhat more consistent and productive of greater immunologic deficiency in the mouse than in the rabbit. This result is probably due to the greater immunologic maturity of the rabbit than the mouse at birth (33).

Further, thymectomy in the early neonatal period will render mice capable of accepting skin homografts across both weak, (H-1) or (H-3), and strong (H-2) histocompatibility barriers, and tumor homografts across strong histocompatibility barriers, after the animals have reached maturity. Neonatal thymectomy also makes it possible for female mice of the $C_{57}Bl$ strain to accept male skin isografts. These findings, together with the observation reported herein that skin homotransplantation is not facilitated by neonatal thymectomy in the rabbit, indicate further that mice, being less mature immunologically at birth than are rabbits, represent more ideal animals in which to study the role of the thymus in development of immunologic potential.

Consideration of the results reported herein and those of others indicating that thymectomy at birth in rabbits (18), mice (20, 21), and rats (40, 41) interferes with development of full immunologic capacity, when viewed in light of the observations of Glick *et al.* (16) and Mueller *et al.* (17), is most provocative. The latter observers found that removal of the bursa of Fabricius in newly hatched chicks or prevention of development of this organ by hormonal treatment interferes with full development of immunologic potential in these animals. The bursa of Fabricius in the chick, like the thymus in the mammal, is a lymphoid organ which originates as an endodermal-epithelial outpouching, is of maximal relative size in the newly hatched animal, and becomes spent and of lesser significance as the animal approaches maturity. Both of these lymphoid-epithelial organs appear to play key roles in development of full immunologic capacity in their respective hosts. Studies by Papermaster *et al.* (42, 43), in these laboratories, which indicate that bursectomy in the newly hatched chick does not completely abolish immunologic potential in the adult animal but rather produces a striking quantitative reduction insufficient to eliminate the homograft reaction, are consonant with the observations reported herein. It can be seen in our data that thymectomy in the rabbit which reduces but does not completely eliminate antibody response to serum protein antigens and bacteriophage T_2, does not permit homotransplantation in the adult animals. By contrast, thymectomy in the neonatal mouse may abolish completely the capacity to form antibody to bacteriophage T_2 and permit homotransplantation of skin and tumors. Histologic studies of the neonate, reported in part in this communication, may explain these observations. The mouse, at birth, has nearly all of its true lymphoid tissue limited to the thymus whereas, in the rabbit and in the chicken other organs possess lymphoid tissue at birth or hatching (44). The failure of thymectomy in newly hatched chicks to alter the immunologic potential of the maturing animal (44) probably only reflects the vigorous participation of the bursa of Fabricius in the development of full immunologic capacity.

The studies reported herein also demonstrate that in mice thymectomized at birth the cells of the peripheral lymphoid tissues, spleen and lymph nodes, lack immunologic capacity even when introduced into the intact F_1 hybrid host equipped with a normal thymus, as indicated by failure to induce runt disease or Simonsen's splenic enlargement.

It is, indeed, tempting to interpret all of our findings as strong support for the postulate, perhaps first expressed by Ruth (45), and more recently elucidated by Auerbach (46), that the thymic cells are distributed centrifugally to the spleen and other "peripheral" lymphoid tissues where they play an essential role in immunologic processes. Particularly in the light of Porter and Cooper's (47) recent communication, establishing that small lymphocytes from the thoracic duct can seek out an environment in spleen, lymph nodes, and bowel

wall in which to develop into larger, immunologically competent, pyroninophilic cells, it seems most likely that the thymus functions as a "fertile crescent" from which lymphoid cells of epithelial origin are distributed centrifugally to the spleen, lymph nodes, bowel, and other lymphoreticular sites, where they settle down, perhaps mature, and function as the immunologically competent cells responsible for adaptive immunity. In this regard, and considering their ultimate origin in epithelium, it is tempting to consider these cells as transposed epithelial elements capable of specialized adaptive secretory activity (antibody synthesis) even in their new locations. The similarity of cytoplasmic ultrastructure of antibody-producing cells and pancreatic cells has already occasioned considerable concern (48).

Certainly other possibilities exist by which the thymus might function to play an essential role in development of full immunologic potential. Among the possibilities that occur to us, and which we feel must be investigated with our favored hypothesis expressed here, is the production by the thymus of a hormone or hormones essential to normal growth, maturation of cells, and normal function of lymphoid tissue. Still another attractive possibility is that thymic cells operate in the transmission of messages, perhaps *via* nucleic acid transfer, through the agency of a nurse cell (Nährmutterzellen) function (49) to cells of mesenchymal origin, thus instilling immunologic capacity.

Whatever the role of the thymus and the bursa of Fabricius may be, it is clear that they have a key role in development of the immunologic system in mammals and chickens. Further, experiments presented here provide evidence that, whatever this function, it is not limited strictly to the neonatal period, since in C_3H mice skin homografts from Ce mice will subsequently be accepted even when the animals are thymectomized as late as 30 days of age.

One means of analyzing the role of the thymus in development of immunologic capacity and the development of the remainder of the lymphoid tissue might be to couple the ontogenetic analysis of development of lymphoid tissue and immunologic capacity with a critical phylogenetic study. Subsequent papers in this series will present the results of this method of analysis.

SUMMARY

In rabbits, complete thymectomy before the age of 5 days produced immunologic deficiency in the adult animals, as indicated by reduced antibody production to bovine serum albumin and bacteriophage T_2. Homotransplantation immunity was unaffected, however.

In an inbred strain of mice, complete neonatal thymectomy resulted in complete inability of the 60-day-old animals to form antibody to bacteriophage T_2.

Inbred mice, completely thymectomized at birth, had a deficient homograft response, indicated by acceptance of skin homografts from strains differing in both the weaker and stronger (H-2) histocompatibility antigens. Tumor trans-

plants (mammary adenocarcinoma) were also successful across the H-2 genetic barrier in mice thymectomized at birth. Neonatal thymectomy also eliminated the Eichwald-Silmser phenomenon, rendering female mice capable of accepting isografts of male skin.

Transplantation immunity in mice was also affected by later thymectomy, at 30 days of age, in certain strain combinations involving weak histocompatibility differences.

Spleen and lymph node cells from mice thymectomized at birth or at 6 days of age, and sacrificed 2 months later, did not produce a graft *versus* host reaction in appropriate F_1 hybrid recipients, indicating that such cells are immunologically inactive.

Neonatal thymectomy of F_1 hybrid mice, and in one strain combination thymectomy at 40 days of age, produced animals with inordinate susceptibility to runt disease (homologous disease) following injection of parent strain spleen cells 35 days (neonatal surgery) and 10 days (surgery at 40 days) later.

Mice thymectomized at birth also showed growth failure and were short-lived.

Studies of newborn mice indicated that they have true lymphocytes only in the thymus, and lack such cells in the spleen, lymph nodes, and gut. In normal mice, adult lymphoid structure develops gradually, beginning during the 1st week of life and continuing for the next month. In contrast, mice thymectomized at birth do not develop mature lymphoid structure: the lymph nodes and spleens tend to be small and poorly organized, and show a quantitative deficiency in lymphoid cells.

It is our current working hypothesis that the thymus makes a major contribution toward the centrifugal distribution of lymphoid cells which, in turn, is essential to the full expression of immunologic capacity.

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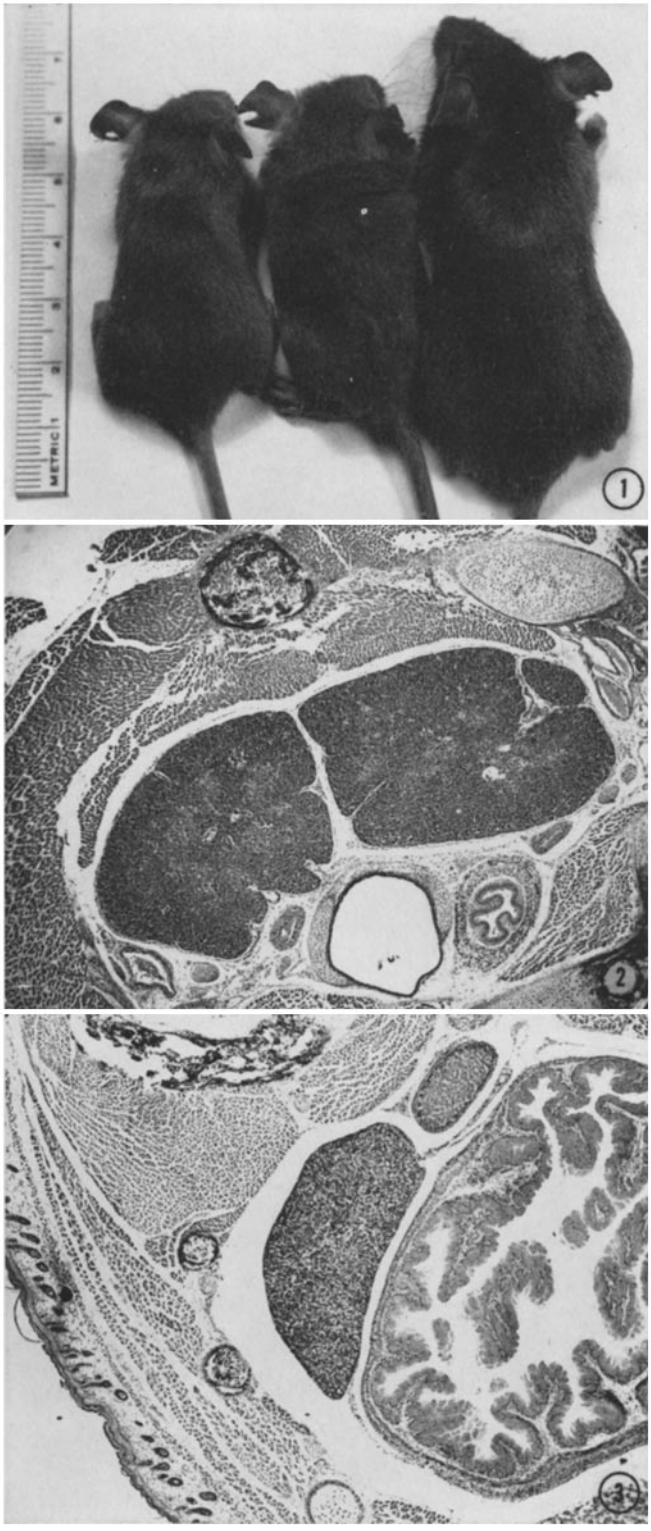
EXPLANATION OF PLATES

PLATE 104

FIG. 1. A comparison, at 60 days of age, of mice thymectomized at birth and a sham-operated litter mate. Notice the runting of the thymectomized animals and the roughening of their fur. Development of diarrhea and early death characterize these mice.

FIG. 2. Low power view of the thymus in a newborn mouse. Note the development of the cortex and medulla, and the dense cellularity of the thymus at this age. Hematoxylin and eosin. × 30.

FIG. 3. Low power view of spleen and intestine of the newborn mouse. Note the lack of development of red and white pulp in the spleen. Lymphoid tissue is virtually lacking in the spleen at this age. No lymphoid tissue is to be found in the gut at this time. Hematoxylin and eosin. × 30.



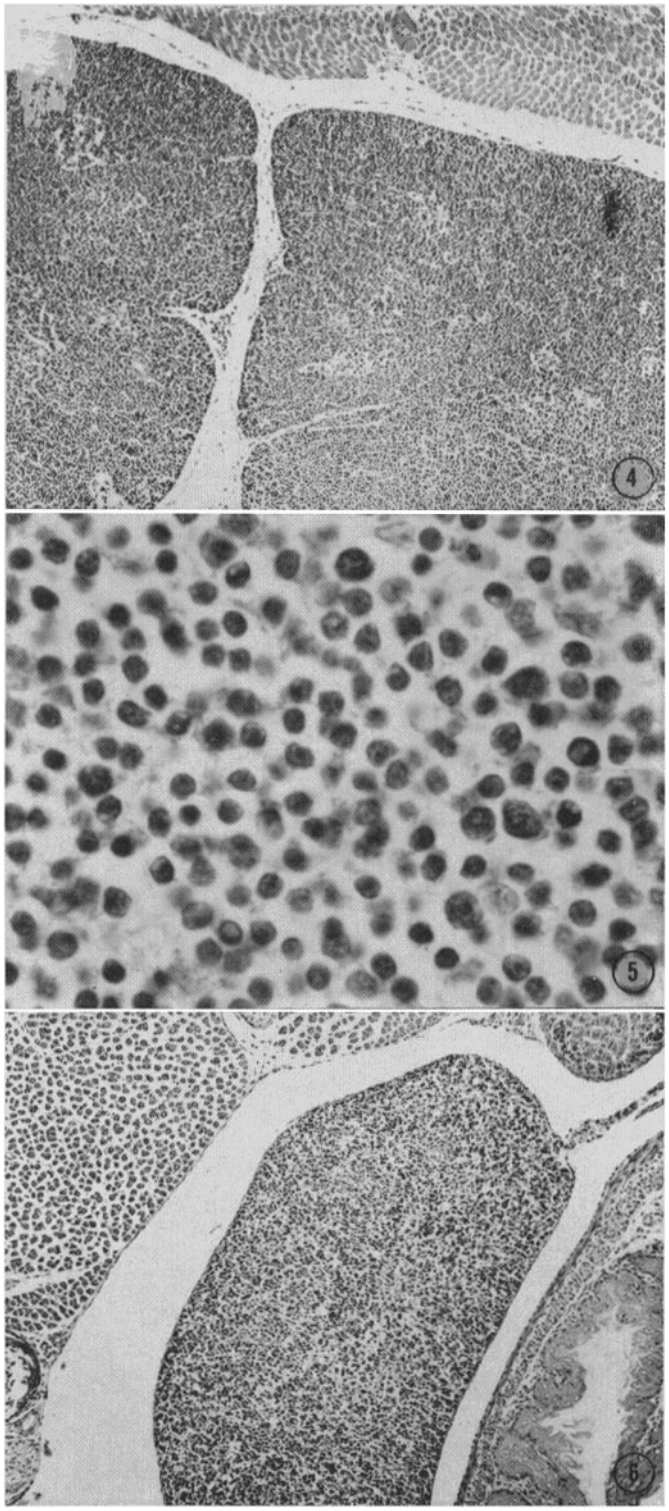
(Good *et al.*: Thymus in development of immunologic capacity)

PLATE 105

FIG. 4. Medium power view of the thymus of a newborn mouse. Note the cellular structure; the great majority of these cells are thymocytes or small lymphocytes. Hematoxylin and eosin. × 115.

FIG. 5. High power view of thymus cells in a newborn mouse. The cell population is composed of small lymphocytes (thymocytes) and epithelial stromal cells. Hematoxylin and eosin. × 770.

FIG. 6. Medium power view of the spleen of a newborn mouse. Note the relatively sparse cellularity and the almost complete lack of adult organization. At this stage the spleen is primarily reticular, erythropoietic, and myelopoietic; very little, if any, lymphopoiesis may be seen. Hematoxylin and eosin. × 115.



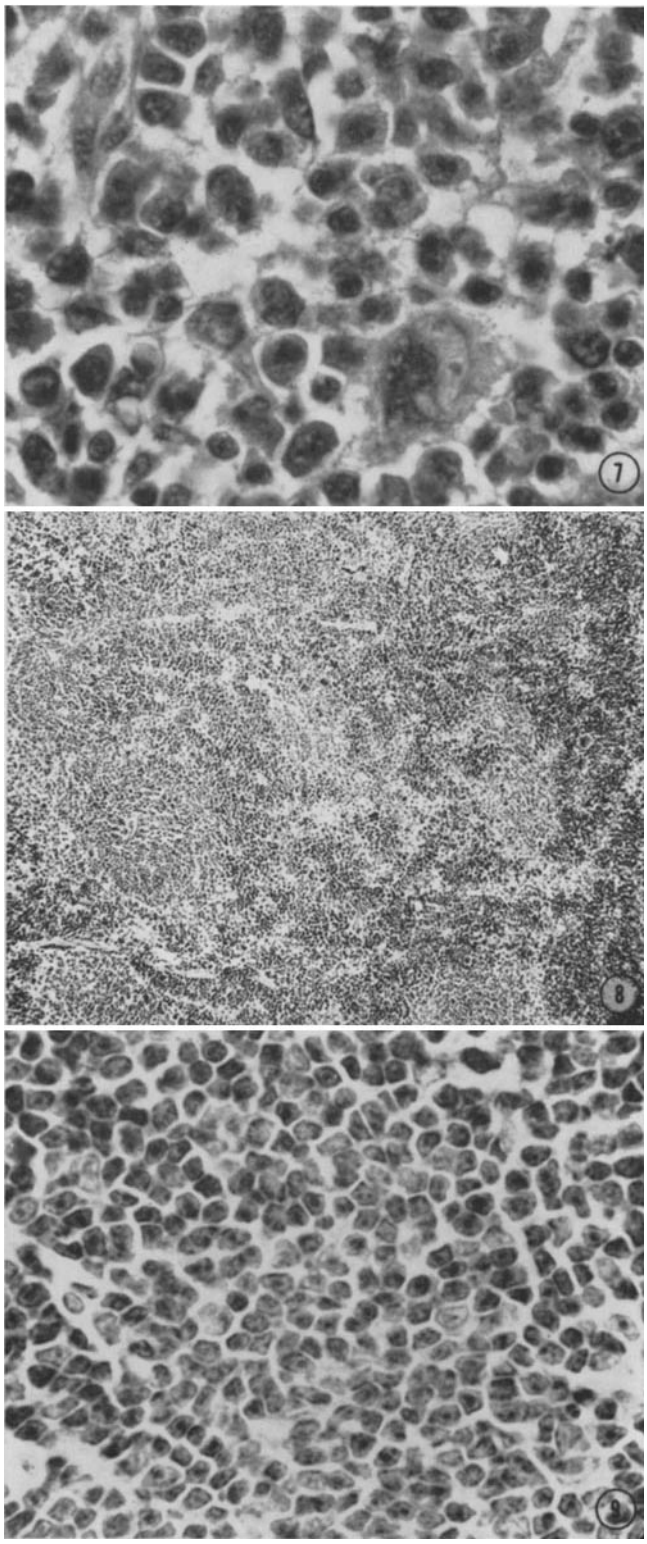
(Good *et al.*: Thymus in development of immunologic capacity)

PLATE 106

FIG. 7. High power view of a representative area of spleen from a newborn mouse. Notice that most of the cells are reticulum cells, myeloid cells, and erythropoietic cells. Very few lymphocytes are present, and no evidence of lymphopoiesis is observed. Hematoxylin and eosin. × 760.

FIG. 8. Medium power view of the spleen from a 40-day-old mouse sham-operated at birth. Note the organization into cortical and medullary portions, and the presence of follicles and lymphopoietic centers. Hematoxylin and eosin. × 75.

FIG. 9. High power view of the spleen from a 40-day-old mouse sham-operated at birth. Note that the majority of cells are small lymphocytes, although some reticulum cells and medium-sized lymphocytes are also present. Hematoxylin and eosin. × 610.

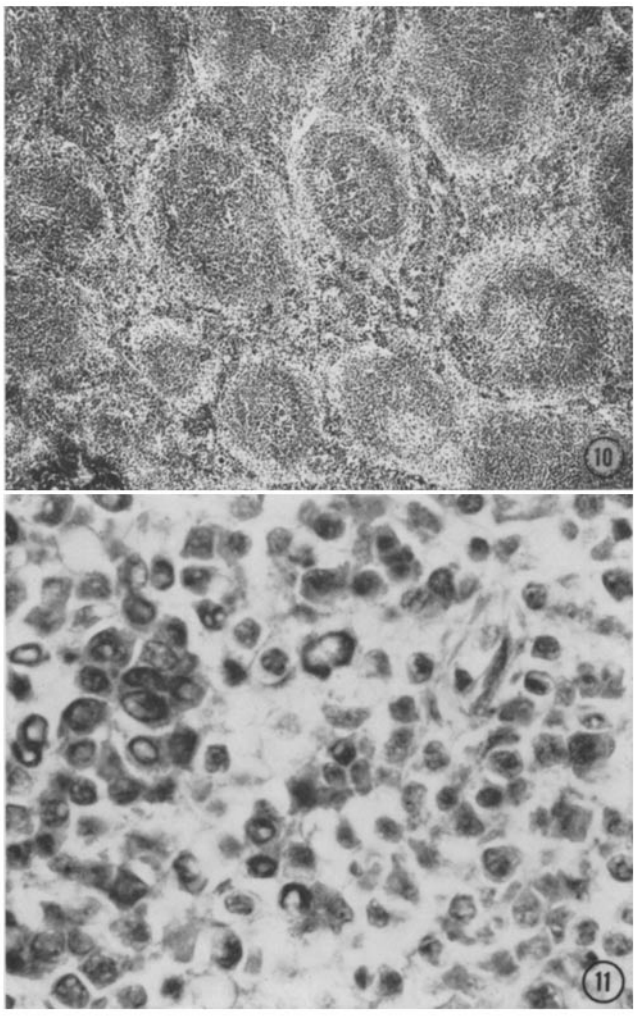


(Good *et al.*: Thymus in development of immunologic capacity)

PLATE 107

FIG. 10. Medium power view of the spleen of a 40-day-old mouse thymectomized on the 1st day after birth. Note the follicular organization. However the periphery of follicles, normally filled with small lymphocytes, are strikingly deficient in these cells. The mantle zones, which normally contain plasma cells, lack these elements. Hematoxylin and eosin. × 75.

FIG. 11. High power view of spleen from a mouse thymectomized at birth. By contrast with Fig. 9, the cells in the spleens of these animals are primarily medium-sized lymphocytes, large lymphocytes, and reticulum cells. Hematoxylin and eosin. × 600.



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