The Effect of Neonatal Thymectomy on *Mycobacterium leprae* Infection in Mice[1, 2]

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It is generally accepted that thymic function is closely associated with the development of immunological capacities such as serum antibody formation, cellular immunity, delayed hypersensitivity and allograft rejection. Removal of the thymus at birth in mice, as well as in other animals, results in a reduction of humoral antibody formation against many antigens ([1, 2, 5, 8, 11, 12, 14-17, 20, 29, 36, 40, 41, 43]). The pool of circulating lymphocytes in neonatally thymectomized mice is deficient not only in its number of cells, but also with respect to the number of antigen-responsive cells ([26]). Neonatally thymectomized rodents also present defective cell-mediated immune responses, such as delayed hypersensitivity reactions ([3, 20]), allograft rejection ([9, 25]) and graft-versus-host reactions ([9]). As a result, neonatally thymectomized animals are incapable of developing adequate resistance to various pathogens. They are highly susceptible to runting disease and acute bacterial infections.

Mice have been widely used as a model to obtain proliferation of *Mycobacterium leprae*. Using inoculation of the mouse foot pad, Shepard ([37]) was the first to achieve success in growing human leprosy bacilli in animals. This has been confirmed by Rees ([31]) and is now a widely employed technic. The growth in the foot pad is self-limited and localized. Such experimental transmission of *M. leprae* was shown by Rees ([31]) and Pattyn and Janssens ([30]) to yield slow but consistent bacterial reproduction until the limit of 10^6-10^7 organisms was reached. Gaugas produced enhanced but still localized growth of human leprosy bacilli in total body (900 r) x-irradiated mice, thymectomized at six weeks of age and transfused with bone marrow cells ([15]), and also in the mice which were thymectomized and injected with antilymphocytic globulin ([16]). Rees and his associates ([33]) reported a systemic infection of *Mycobacterium leprae* in mice which were thymectomized at six weeks of age, total body irradiated with 900 rads, and transfused with syngeneic bone marrow cells. Binford *et al* ([6]) reported a high yield of *Mycobacterium leprae* harvested from mice thymectomized at six weeks of age, irradiated with 900 rads but having one femur shielded by a lead band.

In all the above experiments, mice were thymectomized at the adult stage and the route of infection was either subcutaneous into foot pads, or intravenous. No experiment studying simple neonatal thymectomy and intraperitoneal infection of *M. leprae* in mice has been found. The purpose of the present study was to evaluate in series the harvest of *M. leprae* from major organs of the recticuloendothelial system, i.e., liver, spleen, lungs and kidneys of neonatally thymectomized mice, to determine the extent of such infection and to determine if the lower temperature reflected by the mouse foot pad is indeed necessary for the proliferation of *M. leprae*.

MATERIALS AND METHODS

Experimental animals. Newborn inbred C_3H mice, a strain susceptible to foot pad infection by *M. leprae* ([38]), were used in this experiment. They were divided into three groups: a) neonatally thymectomized (54 animals), b) sham-thymectomized (23 animals), and c) nonthymectomized (21 animals). The mice were nursed by their mothers until one month old. They were then weaned, separated and all groups were fed Purina Chow and drinking water *ad libitum*. The drinking water of all mice had oxytetracycline (oxytetracycline hydro-

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chloride 500 mg per vial, Chas. Pfizer & Co., Inc.) ([18]) added in a concentration of 3 mg/100 ml.

Thymectomy. Thymectomy was performed under a dissecting microscope within 24 hours of birth. The mice were anesthesized by ethyl ether, held steady with masking tape, and an incision was made on the ventral surface of the neck. The submandibular salivary gland was pulled upward to expose the sternal notch, which was then divided along the mid-line for about five millimeters. The two lobes of the thymus were exposed by lifting the upper sternum. A Pasteur pipette connected to a water vacuum pump was used to suck out the thymic tissue. The wound was closed by a single silk suture and sealed with collodion. The sham-thymectomized animals were subjected to an identical operative procedure omitting only the removal of the thymus. The nonthymectomized animals were not operatively manipulated. Both sham-thymectomized and nonthymectomized mice were regarded as control groups. Completeness of thymectomy in each mouse was assessed by autopsy at the time of animal sacrifice and careful histopathologic examination of any suspect tissue in the thymic area. Incompletely thymectomized mice were excluded from the neonatally thymectomized group and pooled to develop a fourth, partially thymectomized group. Eighty-one and a half percent of the thymectomies were thus determined to be complete.

Bacillary inoculum. A leproma was obtained by biopsy from a patient having untreated lepromatous leprosy. It was stored in the freezer for four days before inoculation into the mice. The epidermis was removed first, and the biopsy tissue was minced thoroughly with scissors and ground in 2 ml of Hank's balanced salt solution (BSS) in a motor-driven Duall ground glass tissue grinder. The suspension was centrifuged at 1,500 rpm for five minutes to get rid of tissue debris in order to make a clearer smear for counting. The supernatant was again centrifuged at 5,000 rpm for 20 minutes and the sediment resuspended in 2 ml of Hank's BSS and again centrifuged at 5,000 rpm for 20 minutes.

TABLE 1. *Schedule and number of animals sacrificed.*

	Months					
	1	2	4	6	8	10
Neonatally thymectomized	4	4	4	4	4	4
Sham-thymectomized	3	3	3	3	3	3
Nonthymectomized	3	3	3	3	3	3
Partially thymectomized	2	1	2	1	2	2

The final sediment was resuspended in 1 ml of Hank's BSS and from it a bacillary suspension of 4 x 10^8 bacilli per ml (with 25.8% solid bacillary forms) was prepared. All the mice were inoculated intraperitoneally with 0.1 ml of this bacterial suspension (4 x 10^7 bacilli per animal) within 48 hours after birth. Identical inoculation was repeated twice on alternate days. Thus, each animal received a total of 1.2 x 10^8 bacilli.

Bacillary counting. The number of mice sacrificed periodically are shown in Table 1. The livers, spleens, kidneys and lungs from the sacrificed mice of each experimental group were removed at the time of sacrifice and weighed. Each organ was separately homogenized in 1:5 dilution of Hank's BSS in a motor-driven Duall ground glass tissue grinder. The suspension was centrifuged at 1,000 rpm for five minutes to dispose of tissue debris. The supernatant was again centrifuged at 5,000 rpm for 20 minutes and the sediment resuspended in one milliliter of Hank's BSS and used for the preparation of acid-fast stained smears. Glass slides were cleansed with 5% glacial acetic acid in 95% alcohol. One one-hundredth of each suspension was withdrawn with a Motte pipette and used *in toto* to form a homogeneous smear within a 1 x 2 cm marked out rectangle on a glass slide, dried at room temperature, fixed with heat and stained by the Ziehl-Neelsen procedure. Two smears were made and examined from each suspension of each collected organ from each sacrificed animal.

The average bacillary count of 200 to 600 fields, each 0.007 mm^2, was determined utilizing a calibrated ocular under oil immersion. Both the total number of bacilli and the number of solid form bacilli were

counted. From these counts and the known area of the smear, the total number of bacilli and solid-form bacilli per organ were calculated. The acid-fast bacillary suspensions were cultivated in Lowenstein-Jensen's media to rule out the possibility of contamination by other cultivatable acid-fast organisms. Two adult mice were inoculated intraperitoneally with one milliliter of the remaining pooled suspension from all organs and animals of each sacrificial period. These were sacrificed six months later to rule out the possibility that the acid-fast bacilli in the control suspensions might have been *Mycobacterium lepraemurium*.

Histopathologic examination. Portions of spleen, liver, lung, kidney, mesentery, sternum, and abdominal wall skin from a few representative experimental animals were fixed in 10% buffered formalin. Sternums were decalcified before trimming and embedding. The specimens were sectioned and stained with hematoxylin-eosin and Ziehl-Neelsen stains. Some of them were stained with Triff stain ([45]), which is a combined saffron-trichrome and acid-fast stain yielding superior tissue cell differentiation. All of the sections were carefully examined for pathologic changes and for the presence of acid-fast bacilli.

RESULTS

***Mycobacterium leprae* harvest from liver, spleen, lungs and kidneys of infected mice.** The pooled bacterial count for each animal from livers, spleens, lungs and kidneys is shown in Table 2 and Figure 1. The average total pooled-organ bacterial count of neontally thymectomized mice increased from 9.9×10^6 in the first month to 2.2×10^8 in the fourth month, and reached a peak of 3.0×10^8 in the sixth month. Then the number decreased to 3.7×10^7 in the eighth month, and dropped to 6.5×10^5 in the tenth month. The average pooled-organ solid-form (viable) bacterial count increased from 3.0×10^5 in the first month to a peak of 1.3×10^8 in the fourth month and 1.2×10^8 in the sixth month. The number decreased to 3.2×10^6 in the eighth month, and to 0.5×10^5 in the tenth month. In contrast, the average total pooled-organ bacterial count of sham-thymectomized mice decreased gradually from 3.8×10^7 in the first month to 8.9×10^5 in the eighth month, and bacilli were no longer detectable in the tenth month. The number of solid-form bacilli, likewise, decreased from 8.0×10^6 in the first month to 0.4×10^5 in the eighth month. The nonthymectomized

TABLE 2. *Pooled total and solid-form bacterial counts.*

	1 month	2 months	4 months
Tx (t)	$9.9(\pm1.9) \times 10^6$	$2.5(\pm0.5) \times 10^7$	$2.2(\pm0.3) \times 10^7$
(s)	$3.0(\pm1.2) \times 10^6$	$9.4(\pm3.4) \times 10^6$	$1.3(\pm0.2) \times 10^7$
Sx (t)	$3.8(\pm1.9) \times 10^7$	$3.6(\pm2.2) \times 10^7$	$8.9(\pm3.6) \times 10^6$
(s)	$8.0(\pm2.4) \times 10^6$	$4.9(\pm2.1) \times 10^6$	$8.9(\pm3.8) \times 10^5$
Nx (t)	$3.7(\pm2.0) \times 10^7$	$3.3(\pm2.3) \times 10^7$	$1.0(\pm0.5) \times 10^7$
(s)	$8.1(\pm3.9) \times 10^6$	$5.9(\pm3.9) \times 10^6$	$1.3(\pm0.3) \times 10^6$
	6 months	8 months	10 months
Tx (t)	$3.0(\pm1.2) \times 10^8$	$3.7(\pm1.2) \times 10^7$	$6.5(\pm2.5) \times 10^5$
(s)	$1.2(\pm0.2) \times 10^8$	$3.2(\pm1.3) \times 10^6$	$0.5(\pm0.5) \times 10^5$
Sx (t)	$4.9(\pm1.7) \times 10^6$	$8.9(\pm2.1) \times 10^5$	0
(s)	$4.1(\pm1.5) \times 10^5$	$0.4(\pm0.7) \times 10^5$	0
Nx (t)	$4.1(\pm2.0) \times 10^6$	$9.3(\pm4.3) \times 10^5$	0
(s)	$4.4(\pm2.5) \times 10^4$	$0.5(\pm0.8) \times 10^5$	0

Tx: neonatally thymectomized group.
Sx: sham-thymectomized group.
Nx: nonthymectomized group.
(t): total bacterial count (including solid and granular forms).
(s): solid-form bacterial count.
The figures in parentheses are expressed as ± standard deviation.

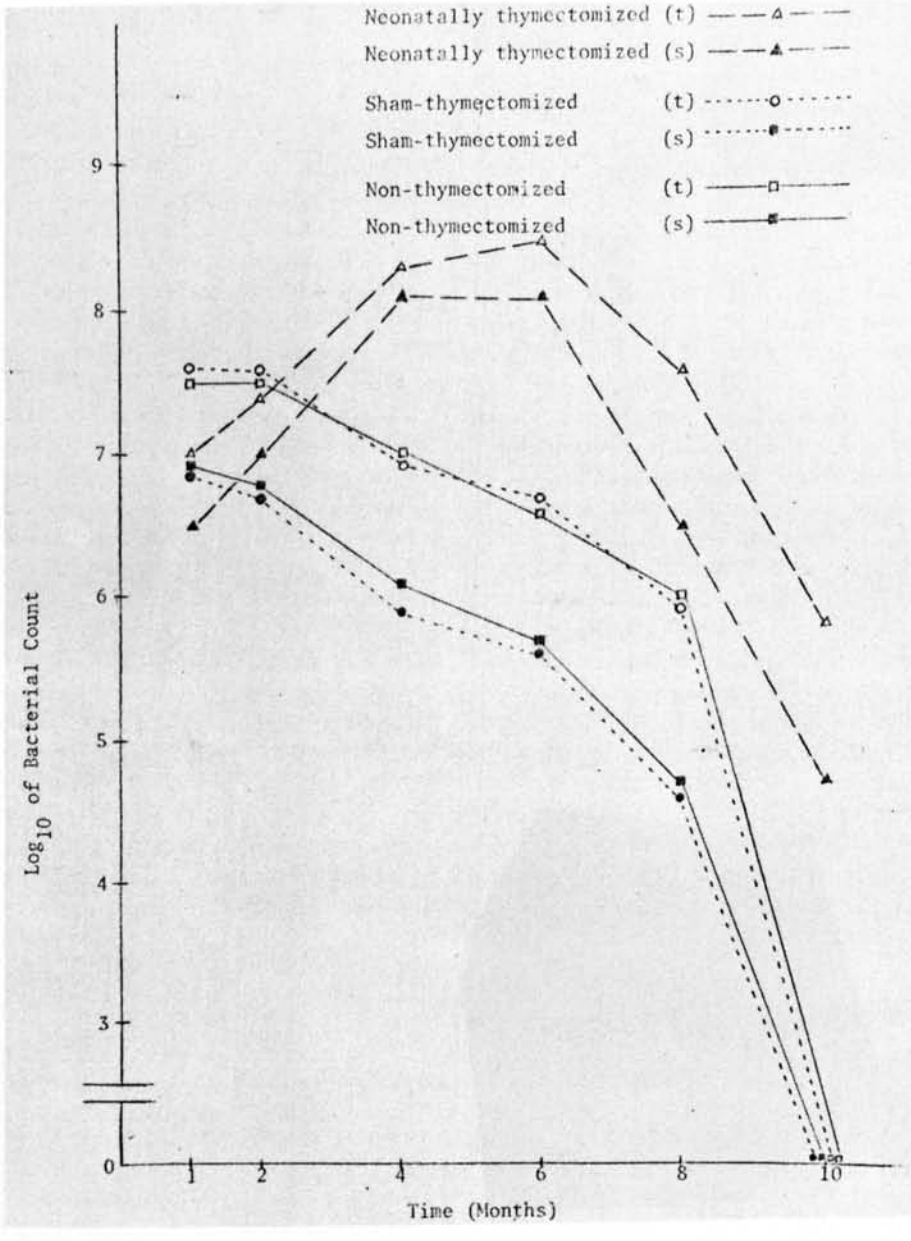

FIG. 1. Pooled-organ total (t) and solid-form (s) bacterial counts.

group yielded results very similar to those of the sham-thymectomized group with respect to both total counts and solid-form counts. All differences in counts between the sham-thymectomized and the nonthymectomized groups were statistically nonsignificant. The greatest difference in bacterial count between the thymectomized and the control groups was in the sixth month. At this time, the total pooled-organ bacterial count of the thymectomized group was 61 times greater than that of the sham-thymectomized group and 73 times greater than that of the nonthymectomized group. The solid-form count of the thymectomized group was 293 times greater than that of the sham-thymectomized group and 273 times greater than that of the nonthymectomized group. The differences in total and solid-form bacterial counts between

the neonatally thymectomized and the sham-thymectomized groups were statistically highly significant ($p < 0.01$) in the fourth, sixth and eighth months. Nevertheless, the differences in solid-form bacterial count in the second and tenth months were

TABLE 3. *Percentage of solid-form bacilli relative to total bacillary count.*

	Months					
	1	2	4	6	8	10
Tx	30.3%	37.7%	59.1%	40.0%	8.6%	7.7%
Sx	21.0%	13.6%	10.0%	8.4%	4.5%	0/0
Nx	21.9%	17.9%	13.0%	10.7%	5.6%	0/0

Tx: neonatally thymectomized group.
Sx: sham-thymectomized group.
Nx: nonthymectomized group.

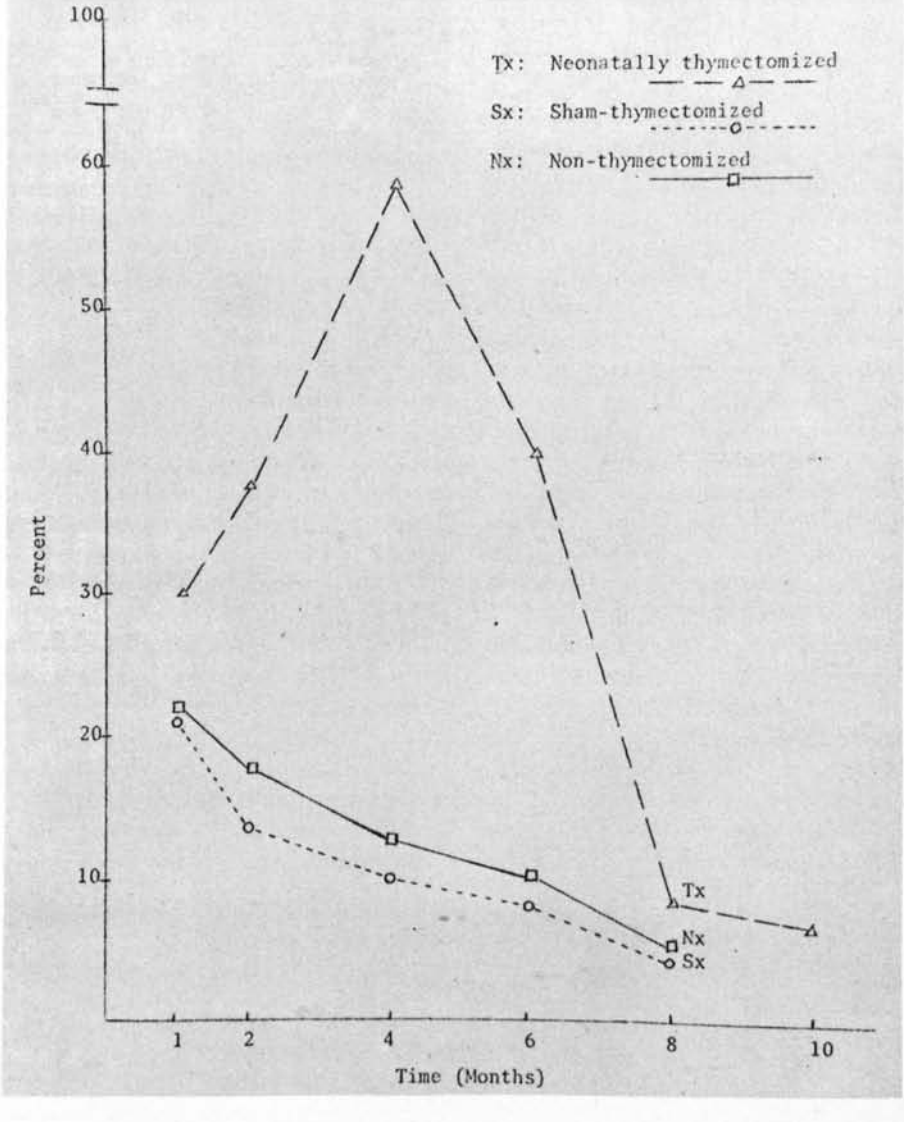

FIG. 2. Percentage of solid-form bacilli relative to total bacillary count.

nonsignificant ($p > 0.10$). The rest were significant, with $p < 0.05$. The partially thymectomized group yielded bacterial harvest determinations intermediate between the thymectomized and control groups, and did not yield any significant conclusions.

The percentage of solid-form bacterial count relative to the total bacterial count (including solid and segmented forms) of the three groups of mice is shown in Table 3 and in Figure 2.

In the neonatally thymectomized group, the percentage of solid-form to total bacillary count increased from 30.3% in the first month, to a peak of 59.1% in the fourth month, and decreased to 40.0% in the sixth and 7.7% in the tenth month. In the sham-thymectomized group, however, the percentage of solid forms showed a steady decrease from 21.0% in the first to 4.5% in the eighth month. The nonthymectomized group showed a similarly steady decline from 21.9% in the first of 5.6% in the eighth month. The greatest percentage difference between thymectomized and control groups was in the fourth month, in which the neonatally thymectomized group was 5.9 times and 4.6 times higher than the sham-thymectomized and nonthymectomized groups respectively.

Effect of oxytetracycline on "runting." The mortality rates in the different groups of mice are shown in Table 4. Within the first two days after operation, 10 of 54 (18.5%) thymectomized mice and 2 of 23 (8.7%) sham-thymectomized mice died. No death occurred in the nonthymectomized group during this period. During the ten month period of observation, an additional 8 mice of the remaining 44 (18.2%) neonatally thymectomized mice died. In the same period, 1 of 21 (4.8%) sham-thymectomized mice and 1 of 20 (5.0%) nonthymectomized mice died of pneumonia. The body weights and probable causes of death of the eight thymectomized mice are shown in Table 5. The cause of death was determined by observation of any gross pathologic change at autopsy and microscopic examination of any suspect pathologic tissue. Thus, "wasting" occurred in only 6 of 44 mice.

Three female thymectomized mice were left over when the experiment ended after the tenth month. One of these female mice developed a tumor at the age of nine months and died at the age of eleven months of an absecessed cartilage-containing neoplasm. Another neonatally thymectomized mouse developed a rapid growing adenocarcinoma of the breast at the age of thirteen months and died within one month. One was alive and well at 18 months. One male and one female sham-thymectomized mouse were left over at the end of ten months. The female mouse developed adenocarcinoma of the breast at age of fourteen months and died in one and a half months. The male mouse survived in health as did one left over female nonthymectomized mouse.

The neonatally thymectomized mice weighed less than the sham-thymectomized and nonthemectomized mice until the fourth month. After the sixth month, the body weights of all three groups were essentially the same.

Histopathologic examination. At the age of two months, acid-fast bacilli were found only within a few Kupffer cells and in tissue macrophages of the spleen, lungs and kidneys in the neonatally thymectomized mice. More phagocytic cells in these organs of the sham-thymectomized and nonthymectomized mice contained acid-fast bacilli. The spleens of the thymectomized mice showed poorly developed follicles or germi-

TABLE 4. *Mortality rates.*

	Death due to operation[a]		Death not due to operation[b]	
Tx	10/54	(18.5%)	8/44	(18.2%)
Sx	2/3	(8.7%)	1/21	(4.8%)
Nx	0/20	(0%)	1/20	(5.0%)

[a] In mice dying within 48 hours of operation, death was regarded as due to the operative procedure.
[b] Deaths due to pneumonia and/or pulmonary hemorrhage, gastroenteritis or from unrecognized causes.

TABLE 5. *Body weight and cause of death in neonatally thymectomized mice dying after the second post-operative day.*

Autopsy no.	Age	Body weight (grams)	Cause of death	Average weight of sham-thymectomized mice of same age (grams)
1	10 days	4.1	Pneumonia	5.9
2	10 days	4.8	Pneumonia	5.9
3	12 days	5.0	Gastroenteritis	6.8
4	15 days	5.4	Pneumonia & pulmonary hemorrhage	7.1
5[a]	16 days	7.0	Unrecognized	7.2
6	21 days	7.3	Pneumonia & gastroenteritis	11.9
7	45 days	11.2	Pneumonia & gastroenteritis	19.2
8[a]	50 days	18.5	Unrecognized	20.3

[a] Numbers 5 and 8 showed no sign of wasting disease. The other six showed one or more of the following symptoms in addition to loss of body weight: listlessness, ruffled hair, diarrhea, shortness of breath and loss of appetite.

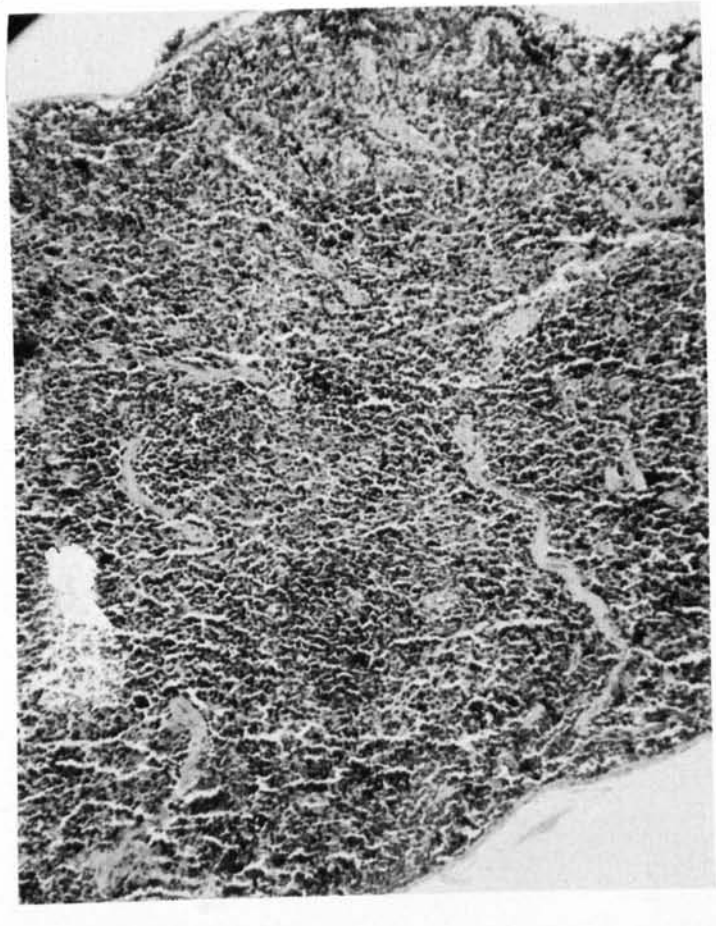

FIG. 3. Spleen of two month old neonatally thymectomized mouse showing poor development of splenic corpuscles, few lymphocytes and relative hyperplasia of reticular cells. Triff stain (60X).

nal centers with relatively few lymphocytes and relative hyperplasia of reticular cells as shown in Figure 3. The histologic appearances of the organs of sham-thymectomized and nonthymectomized mice were essentially the same at every age. Spleen of a two month old sham-thymectomized mouse is shown in Figure 4. They had well-developed follicles and germinal centers with profuse lymphocytes in their spleens by the age of two months. By the age of four and six months, more *M. leprae* were evident within more phagocytic cells in the thymectomized animals than in the other groups. The numbers, however, never exceeded five bacilli per phagocyte and there was no leproma formulation, as described by Rees ([35]) in experimental leprosy and as found in human leprosy ([39]). Acid-fast bacilli were found in some sections of mesentery, rarely in femoral lymph nodes and sternum, and were not found in nerves or skin. Sham-thymectomized and nonthymectomized mice of the same age revealed less acid-fast bacilli in the phagocytic cells of livers, spleens, lungs and kidneys. Occasionally acid-fast bacilli were found in the mesentery. Bacilli were not found in other organs. Bacilli were not detectable in lungs or kidneys of control mice after the age of six months. The spleens of thymectomized, four month old mice showed some follicles and many mature lymphocytes, but the numbers were still less by comparison than in the sham-thymectomized or nonthymectomized groups. At six months the thymectomized mice revealed well-developed follicles and profuse lymphocytes in their spleens, which showed no difference from the spleens of the other groups. At eight months, acid-fast bacilli decreased markedly in the livers and spleens of the thymecto-

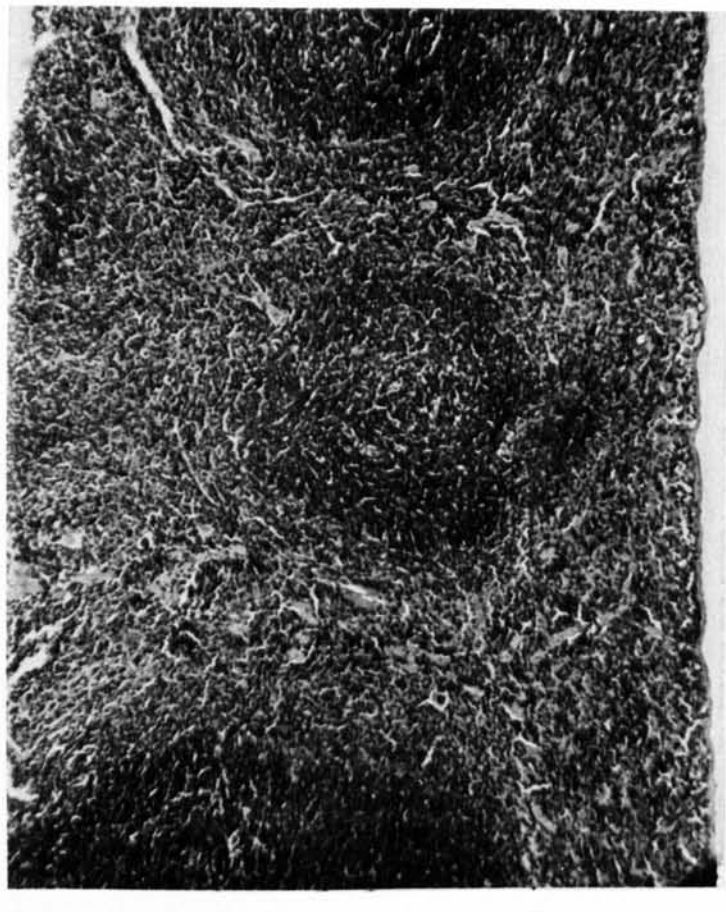

FIG. 4. Spleen of two month old sham-thymectomized mouse showing a well-developed splenic follicle with mature lymphocytes. Triff stain (60X).

mized mice. They were no longer detectable in the kidneys and were only occasionally found in the lungs. At the same age, acid-fast bacilli were only occasionally found in the livers and spleens of the control mice. At ten months of age, no acid-fast bacilli could be found in the lungs or kidneys of any group of mice, but a very few were still detectable in the livers and spleens of thymectomized mice. The spleens of thymectomized mice showed no morphologic difference at eight and ten months from those of the sham-thymectomized or nonthymectomized mice.

DISCUSSION

The total pooled-organ bacterial counts of neonatally thymectomized mice were higher at all experimental periods than in sham-thymectomized or nonthymectomized mice, as shown in Table 2 and Figure 1. From the results presented in Table 2 and Figure 1, the pooled *M. leprae* harvest from livers, spleens, lungs and kidneys of the six month old thymectomized group was 61 times and 73 times higher than similar counts from the sham-thymectomized and nonthymectomized groups respectively, and was 293 times and 273 times higher than the same two control groups with respect to the solid-form bacterial count. The differences were statistically highly significant ($p < 0.001$). This indicates that neonatal thymectomy impairs the immunologic capacity of the host to limit *M. leprae* proliferation. This is in agreement with the findings of Takeya *et al* [42] with respect to *M. tuberculosis hominis* infection in neonatally thymectomized mice, and of Fieldsteel *et al* [13], who demonstrated enhanced *M. leprae* infection in neonatally thymectomized rats, with or without anti-lymphocytic serum treatment.

The results presented in Table 3 and Figure 2 indicate that the percentage of solid-form bacilli was much higher in the thymectomized group than in the two control groups. The solid-form bacilli of *M. leprae* are generally held to be viable [10, 31, 44]. The peak of solid-form bacilli in neonatally thymectomized mice was in the fourth month. It is interesting to notice that the percentage of solid-form relative to total bacterial count increased from 30.3% in the first month to a peak of 59.1% in the fourth month and decreased to 40.0% in the sixth month and 7.7% in the tenth month. Thus, the percentage increase in "viable" bacilli in thymectomized animals supports the conclusion that deficiency of the immune system in the early months is reflected in inability to kill the bacilli. The proliferation of viable bacilli was arrested between the fourth and sixth month, and the bacilli were destroyed in great numbers by reconstituting immunologic mechanisms. The solid-form bacterial count showed no increase from the fourth to sixth month (1.3×10^8 and 1.2×10^8) but a rapid decline to 3.2×10^6 was observed in the eighth month. The spleen of thymectomized mice revealed poorly developed splenic corpuscles and few lymphocytes when they were two months old, similar to the findings of six week old thymectomized mice by Miller [23], but showed some well-developed follicles and an increase in lymphocytes in the fourth month, and showed fully developed follicles and profuse lymphocytes in the sixth month. All of these facts help to support the view that the neonatally thymectomized mice recovered their immunologic capacity to limit the growth of *M. leprae* between the fourth and sixth months. This is in agreement with Rogister [36], who demonstrated that the lymphocyte count of neonatally thymectomized A.SW. mice remained low during the 120 days following thymectomy, but after 150 days, the count rose and became similar to that of normal mice. The mechanism of this recovery is not clear. Kisken *et al* [21] developed the technic of intrauterine thymectomy in the rabbit. By the 24th day of gestation in rabbits, the thymus is the only lymphoid organ in the body of the rabbit. From the 28th day on, four days before birth, there is development of the secondary lymphoid tissue. Thus, it is probable that in C_3H mice the thymus has already influenced the secondary lymphoid organs through thymosin [4] or by prior thymocyte distribution in the fetal stage, and this may be the basis for post-thymectomy immunologic recovery.

It is widely held that a low tissue tem-

perature is a critical requirement for the growth of *M. leprae*. Brand [7] postulated that the higher degree of nerve and other tissue damage in leprosy patients occurs in the cooler areas of the body, and that the predominant distribution of leprosy lesions in cool areas, such as the extremities, results from such low temerature growth requirements. The same reasoning lies behind the employment of the foot pad (temperature 29.95 ± 0.92°C [35]) as the site of mouse inoculation in Shepard's mouse foot pad model. The fact that significant increase in solid-form bacilli occurred in the warmer viscera of mice during the period of immunologic deficiency occasioned by thymectomy, suggests that low temperature is not an obligate factor in the growth of *M. leprae*. Rees [31, 34] reported that if 10^4 *M. leprae* were inoculated into normal CBA mouse foot pads, about 10^6 acid-fast bacilli could be recovered in six to eight months. The number of bacilli declined after eight months and the proportion of degenerate forms increased. The yield of bacilli increased again and reached a second peak in 13 months, but rarely exceeded 10^7. After this, the number declined again and the proportion of viable forms decreased greatly. These findings are similar to the pooled-organ viable form counts in the present experiments in that proliferation of *M. leprae* occurred in the first several months and was limited after six or eight months (Figs. 5 and 6). Since the limitation of bacillary growth in these experiments is apparently due to restored immunologic capacity, the limitation of growth in the foot pads of normal mice may be due to immunologic enhancement built up in response to *M. leprae* infection.

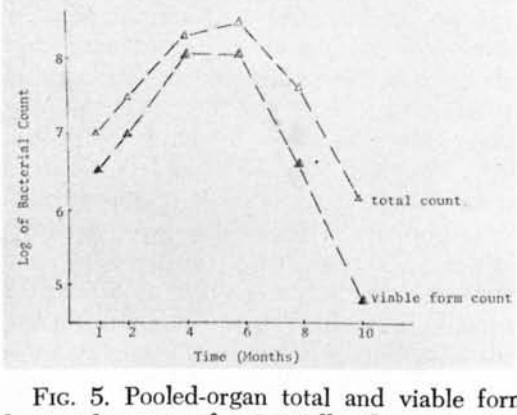

FIG. 5. Pooled-organ total and viable form bacterial count of neonatally thymectomized mice intraperitoneally infected with *M. leprae*.

"Wasting disease" is a common syndrome which occurs frequently in neonatally thymectomized mice. It has been attributed to a deficiency of immunologically competent cells rendering the mice unable to resist invading pathogens. Pathogen-free [19] or germ-free [22, 27, 46] neonatally thymectomized animals usually do not show signs of wasting although they do appear to have varying degrees of immunologic deficiency [19, 27]. The mice in this experiment were fed oxytetracycline in their drinking water. In the present experiment, the mortality of thymectomized mice during the ten months of the experiment was 18.2% (8 of 44 mice) excluding those animals which died from the thymectomy procedure itself. Six of the forty-four (13.6%) showed wasting. Asanuma *et al* [4] reported wasting and mortality within nine weeks in 75% of the neonatally thymectomized, untreated CBA mice. Others report similarly high incidence of wasting [24, 28, 47]. In comparison with the 13.6% wasting and 18.2% mortality in the present experiments, it seems that treatment with oxytetracycline in a concentration of three milligrams per hundred milliliters of drinking water can decrease the incidence of wasting disease and prolong the lives of neonatally thymectomized mice. It also helps to support the view that wasting disease in neonatally thymectomized animals is attributable, at least in part, to impaired immunologic capacity to resist pathogens.

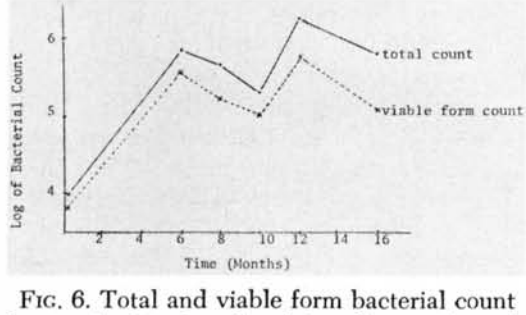

FIG. 6. Total and viable form bacterial count of normal mice inoculated into foot pads and ears with *M. leprae*. Reproduced in part from Rees [34].

SUMMARY

C_3H mice were thymectomized at birth and inoculated intraperitoneally with *Mycobacterium leprae*. The neontally thymectomized and control sham-thymectomized and nonthymectomized mice were sacrificed at one month and bi-monthly through the tenth month. The total acid-fast bacillary content of their livers, spleens, lungs and kidneys were harvested and evaluated with respect to their total numbers and solid-form numbers. Neonatally thymectomized mice had higher total and solid-form bacterial counts than either the sham-thymectomized or nonthymectomized animals but both the total and the solid-form counts decreased after the sixth month. Thus, the animals recovered from the immunologic defect induced by neonatal thymectomy by the sixth month and this recovery is associated with an ability to alter the morphology of the bacilli to a form regarded as nonviable. The recovery of immune capacity was associated with redevelopment of follicles with profuse lymphocytes in the spleens of thymectomized mice after the fourth month. Oxytetracycline in the drinking water, in a concentration of 3 mg per 100 ml, helps to prolong the lives of thymectomized mice, and decrease the incidence of "wasting disease." The significant proliferation of solid-form bacilli in the viscera of the thymectomized mice during their period of immunologic deficiency suggests that the lower tissue temperature postulated as necessary for the success of the proliferation of *M. leprae* in the mouse foot pad may not be an obligate factor.

RESUMEN

A ratones C_3H se les practicó timectomía al nacer y luego fueron inoculados intraperitonealmente con *Mycobacterium leprae*. Los ratones timectomizados neonatalmente, unos de un grupo control en los cuales se simuló una timectomia y unos de otro grupo control que no fueron timectomizados, fueron sacrificados un mes después y luego cada dos meses, hasta el décimo mes. Se recogió el contenido de bacilos ácidoresistentes total de hígado, bazo, pulmón y riñón y se evaluó con respecto a cantidad total y número de formas sólidas. Los ratones timectomizados al nacer tenían recuentos totales y de formas sólidas más altos que los ratones en los cuales se simuló timectomía o aquellos que no se timectomizaron, pero tanto el recuento total como el de formas sólidas disminuyó después del sexto mes. Así, los animales se recuperaron del defecto inmunológico inducido por la timectomía neonatal al sexto mes, y esta recuperación se asoció con una capacidad para alterar la morfología de los bacilos hacia una forma considerada no-viable. La recuperación de la capacidad inmunológica se asoció con un redesarrollo de folículos con gran cantidad de linfocitos en el bazo de los ratones timectomizados, después del cuarto mes. La oxitetraciclina en el agua de beber, a una concentración de 3 mg por 100 ml, ayudó a prolongar la vida de los ratones timectomizados y dismunuyó la frecuencia de "consunción inmunológica" en los animales. La proliferación significativa de bacilos de forma sólida en las visceras de los ratones timectomizados durante su período de deficiencia inmunológica sugiere que la temperatura baja de los tejidos, que se ha postulado como necesaria para obtener una proliferación exitosa del *M. leprae* en la almohadilla de la pata del ratón, puede no ser un factor obligatorio.

RÉSUMÉ

Chez des souris C_3H thymectomisées à la naissance, on a inoculé *Mycobacterium leprae* par voie intrapéritonéale. Les souris thymectomisées à la naissance et les souris thymectomisées témoins, de même que des souris non thymectomisées, ont été sacrifiées après un mois, et ensuite tous les deux mois jusqu'au dizième mois. On a récolté le contenu bacillaire acido-résistant total du foie, de la rate des poumons, et des reins; on a procédé ensuite à une évaluation du nombre total des bacilles et du nombre de bacilles solides. Les souris thymectomisées à la naissance présentatient un nombre total de bactéries de même qu'un nombre de formes solides, plus élevé que les souris-témoins thymectomisées ou les animaux non thymectomisés. Néanmoins, le nombre total de bacilles et le nombre de formes solides diminuait après le sizième mois. Dès lors, on constate que les animaux surmontent le défaut immunologique causé par la thymectomie néo-natale, au sizième mois, cette récupération étant associée avec la capacité d'altérer la morphologie des bacilles en les transformant en une forme considérée comme non viable. La récupération de cette capacité immunitaire était associée avec un nouveau développement des follicules, avec lymphocytes profus, dans la rate des souris thymectomisées, à partis du quatrième mois.

L'oxytetracycline dans l'eau de boisson, à la concentration de 3mg par 100 ml, a aidé à prolonger la vie des souris thymectomisées, et a diminué l'incidence d'une attrition par maladie intercurrente (wasting disease). La prolifération significative de bacilles solides dans les viscères des souris thymectomisées au cours de la période de déficience immunologique, suggère que les températures tissulaires basses, que l'on considère comme un facteur nécessaire pour permettre une prolifération de *M. leprae* dans le coussinet plantaire de la souris, n'est peut être pas un facteur indispensable.

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