BNL 6910

PRIMARY AND SECONDARY ANTITOXIN RESPONSES IN THYMECTOMIZED MICE*

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Submitted: Journal of Immunology

No. of copies: 3

No. of pages: 14

No. of figures: 3

No. of tables: 1

* Research supported by the U. S. Atomic Energy Commission.

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INTRODUCTION

During the past few years renewed interest has been given to the importance of the thymus in immune processes and the possible role of the thymus in the development of immunologic competence. Hammar[1] was the first to study the influence of the thymus on immune reactions in adult animals. Rabbits were thymectomized and immunized with a series of weekly i.v. injections of Salmonella paratyphi B antigen, starting 8 weeks after the operation. Formation of anti-H-agglutinin was observed to be slightly impaired in thymectomized animals as compared to nonoperated controls. The difference in the observed titers, however, was not statistically significant. Similar experiments with thymectomized adult rabbits by Harris et al.[2] and MacLean et al.[3] confirmed the findings of Hammar. Fichtelius et al.[4] studied primary and secondary responses of partially thymectomized adult guinea pigs to immunization with Salmonella typhi. Thymectomized animals showed an impairment of the primary response, whereas no repression of the secondary response could be found. According to Martinez et al.[5] tumor homograft survival was not prolonged in mice thymectomized at the age of 5 to 6 weeks. Thymectomy in rats, 2 to 3 weeks of age, had no or a very slight impedient influence on the Arthus reaction and delayed skin reactions after sensitization with bovine serum albumin[6]. Thymectomized adult guinea pigs showed no inhibition of delayed skin reactions after sensitization with egg-albumin[6].

Great interest has recently been given in study of the effect of thymectomy in new-born animals upon subsequent development of immune mechanisms (reviews 7,8,9). When rabbits were thymectomized at the age of 1-7 days, they showed an impaired ability to form precipitins against bovine serum albumin [6,7,10]. Good et al.[7,11] studied the influence of thymectomy in rabbits up to 3 days old and in mice within 24 hours after birth on formation of neutralizing antibodies

to T_2 coliphage. Antibody formation was reduced in thymectomized rabbits, whereas antibody could not be detected in thymectomized mice[7,11]. Skin and tumor transplantation experiments in rats and rabbits showed that thymectomy in the new-born animal effectively prolonged the survival time of the homograft[5,12,13,14,15]. Postnatal thymectomy also appeared to impede delayed skin reactions after sensitization with bovine serum albumin and to repress the tuberculin sensitivity reaction in rats[6,16].

The present study is concerned with testing the ability of thymectomized mice to produce primary and secondary tetanus antitoxin responses. Mice, thymectomized at time intervals from 1 to 8 days after birth, were given a primary stimulation at the age of 4 weeks and secondary stimulation with tetanus toxoid when 7 weeks old.

MATERIALS AND METHODS

Animals.

Swiss albino mice of an inbred strain were used in these experiments. These mice are specific pathogen-free and have been raised in our colony for about 20 years. All animals were weaned at 4 weeks of age.

Thymectomy.

Thymectomy was performed on half of the animals in each litter. The nonoperated litter-mates served as controls. A total of 68 mice (36 males and 32 females) were thymectomized at the following times after birth; 22 during day one, 21 during day two, 17 on day four and 8 on day eight. The animals were anesthesized with ether and fixed in a supine position on a metal block which was warmed to body temperature. A median incision was made through the skin, sternum or the first three ribs and the underlying fascia. The opening in the thorax was expanded with a small reverse-action wire retractor. Both lobes of the thymus were removed by blunt dissection with a fine wire loop and iris forceps. Dissection was started at the distal poles and care was taken

to avoid rupture of the delicate capsular membrane enclosing each lobe. Bleeding was not usually encountered during this procedure. The incision was closed with four to six single knots of cardiovascular dacron (suture size 6-0). The first suture readjusted the position of the split sternum. The operated site and suture knots were coated with liquid collodion to prevent the mothers from chewing the sutures. Thymectomized animals were returned to the litter immediately after the operation. Operative mortality varied greatly from day to day, with a range of from 10% to 70%. All operated animals were examined macroscopically for completeness of thymectomy or regeneration of thymic tissue when sacrificed for serum at the end of the experiment.

Antigenic stimulation.

All mice were given a primary injection of 0.025 ml aluminum phosphate adsorbed tetanus toxoid (APTT) (Lederle) in each of the hindleg foot pads when four weeks of age. Three weeks later 0.02 ml blood was collected by tail-artery bleeding for titration of antitoxin produced during the primary response. Later in the day all animals were given a booster injection of 0.05 ml fluid tetanus toxoid (FTT) (Lederle) in each of the hindleg foot pads to elicit secondary antitoxin responses. All mice were sacrificed for serum ten days after the second antigenic stimulus.

Tetanus antitoxin titration.

The antibody titer of each serum sample was determined by individual toxin-antitoxin titrations (Ehrlich method) as reported by Hale and Stoner[17]. Titration of the antitoxin produced after primary immunization was performed on whole blood. Previous experiments showed no statistical difference between titers determined on whole blood and titers measured on serum samples obtained

from the same animals[18]. Secondary response antitoxin was measured in serum samples. The tetanus toxin contained 2 x 10^5 M.L.D. per ml, and 1000 M.L.D. were neutralized by 0.025 International Units (I.U.) of tetanus antitoxin.

RESULTS

A comparison of the amount of tetanus antitoxin produced by each thymectomized and nonoperated mouse three weeks after primary stimulation with 0.05 ml APTT is shown in Figure I. The antitoxin titers of one-third of all thymectomized animals were lower than the lowest titer found in non-operated controls. A greater number of animals in the group of mice operated on day 4 showed primary responses comparable to responses of their litter-mate controls than in the other operated and control groups. Antitoxin levels varied considerably in thymectomized mice with a range of 0.0005 to 0.025 I.U. as compared to a range in values in controls of 0.002 to 0.03 I.U. tetanus antitoxin.

Tetanus antitoxin responses obtained after secondary antigenic stimulation with 0.1 ml FTT in thymectomized and nonoperated mice are plotted in Figure II. The ability of thymectomized animals to respond to a secondary stimulus was impaired to a greater extent than the capacity to respond to primary stimulation. In two-thirds of the thymectomized animals, the antitoxin titers were repressed below the lowest titer found in control mice. In contrast to the more varied results observed with primary responses, secondary responses were more uniformly depressed in animals thymectomized on day 1 through day 8. Antitoxin levels ranged from 0.005 to 1.1 I.U. in thymectomized mice and from 0.12 to 5.0 I.U. tetanus antitoxin in nonoperated controls. These findings have been summarized in Figure III. Mean antitoxin titers of thymectomized animals were compared to mean antitoxin titers of their respective litter-mate controls. The primary response of thymectomized animals was

repressed from 20% (mice operated upon on day 4) to 60% (mice operated upon on day 2). The secondary response on the other hand showed an 80% repression in all four experimental groups of thymectomized animals.

A correlation was not observed between the amount of antitoxin produced after primary and secondary stimulation. Several thymectomized animals with fairly good primary responses produced only small amounts of antitoxin after secondary stimulation. Several animals with low primary responses, on the other hand, showed excellent secondary responses. Although a considerable number of operated animals appeared sickly at the end of the experiment (varying degree of inactivity, ruffled fur, and diarrhea in some animals) there was no convincing evidence of a deficiency in the average weight gain in thymectomized mice operated on day 2 through day 8 as compared to litter-mate controls. Animals thymectomized on day 1, however, gained less weight (average) than their litter-mate controls. The difference was statistically significant ($p < 0.05$) (Table 1).

Completeness of thymectomy was judged macroscopically. Small thymic remnants were found in one-third of the animals. No correlation was evident between the amount of thymus present and amount of antitoxin produced. Several animals, judged macroscopically to be completely thymectomized, produced normal amounts of antitoxin. It should also be emphasized that sizable thymic remnants were observed in animals with low titers of antitoxin. No sex difference was apparent in the repressed ability of thymectomized mice to respond to primary and secondary stimulation with tetanus toxoid, although female animals are known to be better antibody producers as male animals[18].

DISCUSSION

The present data indicate that primary responses were not markedly reduced in most mice thymectomized from 1 to 8 days after birth when immunized at the age of 4 weeks with tetanus toxoid. Antitoxin responses after secondary stimulation at the age of 7 weeks were impaired to a greater extent than primary responses. These findings should be stressed since several authors have reported on greatly depressed antibody formation in rabbits and rats,

thymectomized from 1 to 5 days after birth[6,7,10]. No precipitins could be demonstrated in the sera of these animals 10 to 21 days after i.v. or s.c. stimulation with bovine serum albumin. According to Good et al.[7], formation of antibody to T_2 coliphage was almost completely abolished in 8 thymectomized DBA/2 strain mice which had been immunized when 8 weeks of age by i.p. injection of 2×10^{10} particles of bacteriophage. Our results are more compatible with findings in chickens by Warner and Szenberg[19] who demonstrated normal antibody formation against human gamma-globulin in chickens thymectomized within 24 hours after hatching.

It may be stated from the present data obtained in our strain of mice that postnatal thymectomy does not abolish the ability to respond to primary antigenic stimulation at the age of 4 weeks. Special emphasis may be placed on these results since:

a) Primary antigenic stimulation was given before signs of wasting could be noticed in operated animals. All other workers started immunization from 8 to 18 weeks after thymectomy[6,7,10,13,16], i.e. at a period of time during which obvious wasting was reported to occur at least in a few of the animals[6,7,16].

b) The sensitivity of the toxin-antitoxin titration method for measuring antibody is much greater than that of the techniques used by other investigators, e.g. neutralization of T_2 coliphage[7,11], agglutination of tanned red cells[6] or, use of skin homograft rejection as a test for antibody production[12,13,14,15] since with the latter a considerable number of unknown and in part not immunological processes may be involved.

c) A precipitating antibody need not be present in order to measure specific antitoxin since the capacity of antibody to neutralize tetanus toxin is determined with minimal paralysis as the endpoint.

It is of particular interest to notice that secondary responses were markedly repressed at a time interval after operation comparable to that chosen by most other workers[6,7,10,13,16] for primary stimulation. The finding of considerably impaired secondary responses after fairly good primary responses is highly uncommon. In fact we do not know of any agent or procedure that would selectively impair secondary responses to antigenic stimulation. Whole body X- or gamma radiation for instance is known to more effectively repress primary responses than secondary responses after stimulation with tetanus toxoid[20].

An inhibitory effect of postnatal thymectomy on secondary, but not on primary antitoxin responses was an unexpected finding. The hypothesis of a migration from the thymus to other lymphatic organs of immunologically competent stem cells has been considered. Since small lymphocytes appear to be mainly instrumental in primary responses[21], it is unknown at this time why postnatal thymectomy should not produce a corresponding repression of both primary and secondary antitoxin responses. Several alternative explanations for this unusual phenomenon may be considered such as:
a) Wasting might repress the ability to produce antibody to a greater extent than does lack of thymic tissue by itself. Thymectomized animals, immunized at a time when wasting is known to occur, show impaired immune responses, whereas immunization prior to this critical time is followed by almost normal antibody responses. Little is known at present about the pathogenesis of this post-thymectomy wasting syndrome. It should not be excluded that postoperative scarring in the upper mediastinum might contribute to wasting. It is of interest to notice in this respect that postnatal thymectomy in larger animals, such as dogs[22], does not appear to repress the ability of these animals to respond to antigenic stimulation. It remains to be demonstrated whether the late sequelae of postnatal thymectomy may vary from mouse to dog for surgical reasons. In addition,

and perhaps of more importance, the critical time may vary from one species to another when peripheralization of immunologically competent thymic cells occurs[23].

b) Immunologically competent thymic cells peripheralized prior to thymectomy might have reached the end of their life span 4 weeks after birth, i.e. the supply of immunologically competent stem cells might be exhausted. There is no indication in our strain of mice of a marked peripheralization of thymic lymphocytes prior to birth. At birth the thymus is large while mesenteric and other lymph nodes are extremely small. In addition, the concept of peripheralization of immunologically competent thymic cells does not explain why secondary responses should be depressed. It has not been shown beyond doubt that small lymphocytes are instrumental in secondary responses[21]. Experiments are under way to test primary responses of thymectomized mice as a function of time between postnatal thymectomy and antigenic stimulation.

c) Little is known about the possible role in immune mechanisms of substances released from the thymus[7]. It has recently been shown that wasting of thymectomized mice can be prevented by transplantation of homologous thymic tissue, but not by injection of thymic lymphocytes[24]. In the present study, no retardation of growth was noted until 4 weeks after the operation. Before any further conclusions may be drawn concerning the role of the thymus in immune responses, particular consideration must be given to the wasting syndrome, its pathogenesis, and its possible direct or indirect effects on antibody formation in thymectomized animals.

SUMMARY

A total of 68 Swiss albino mice of either sex were thymectomized at the following time intervals after birth: 22 during day one, 21 during day two, 17 on day four and 8 on day eight. Sixty-one nonoperated litter-mates served as controls. All mice were given primary antigenic stimulation with adsorbed tetanus toxoids when 4 weeks of age. Three weeks later a booster injection of fluid tetanus toxoid was given. With these time intervals thymectomized mice showed only slightly impaired primary responses but severely repressed

secondary responses as compared to nonoperated litter-mates. This uncommon finding is difficult to explain at present and results are discussed with regard to other reports on the effect of postnatal thymectomy on immune responses. Special emphasis is placed on the unknown effect of post-thymectomy wasting syndrome.

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FIGURE LEGEND

Figure I. Effect of thymectomy on primary antitoxin responses elicited in mice when 4 weeks of age.

Figure II. Effect of thymectomy on secondary antitoxin responses elicited in mice when 7 weeks of age.

Figure III. Comparison of effect of thymectomy on primary and secondary antitoxin responses in mice.

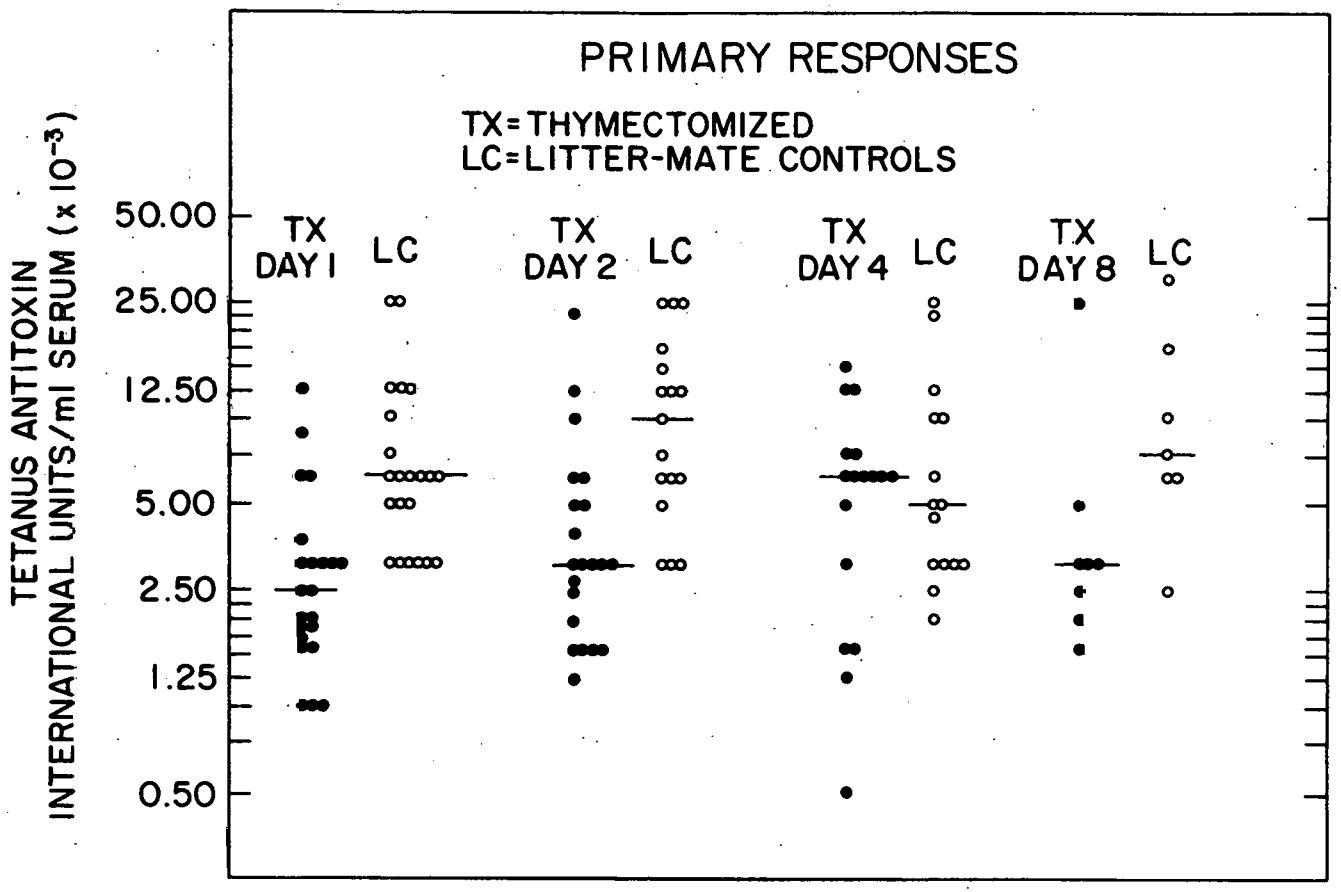
PRIMARY RESPONSES
TX=THYMECTOMIZED
LC=LITTER-MATE CONTROLS
TETANUS ANTITOXIN
INTERNATIONAL UNITS/ml SERUM (x 10⁻³)
50.00
25.00
12.50
5.00
2.50
1.25
0.50
TX
DAY 1
LC
TX
DAY 2
LC
TX
DAY 4
LC
TX
DAY 8
LC

FIGURE 1

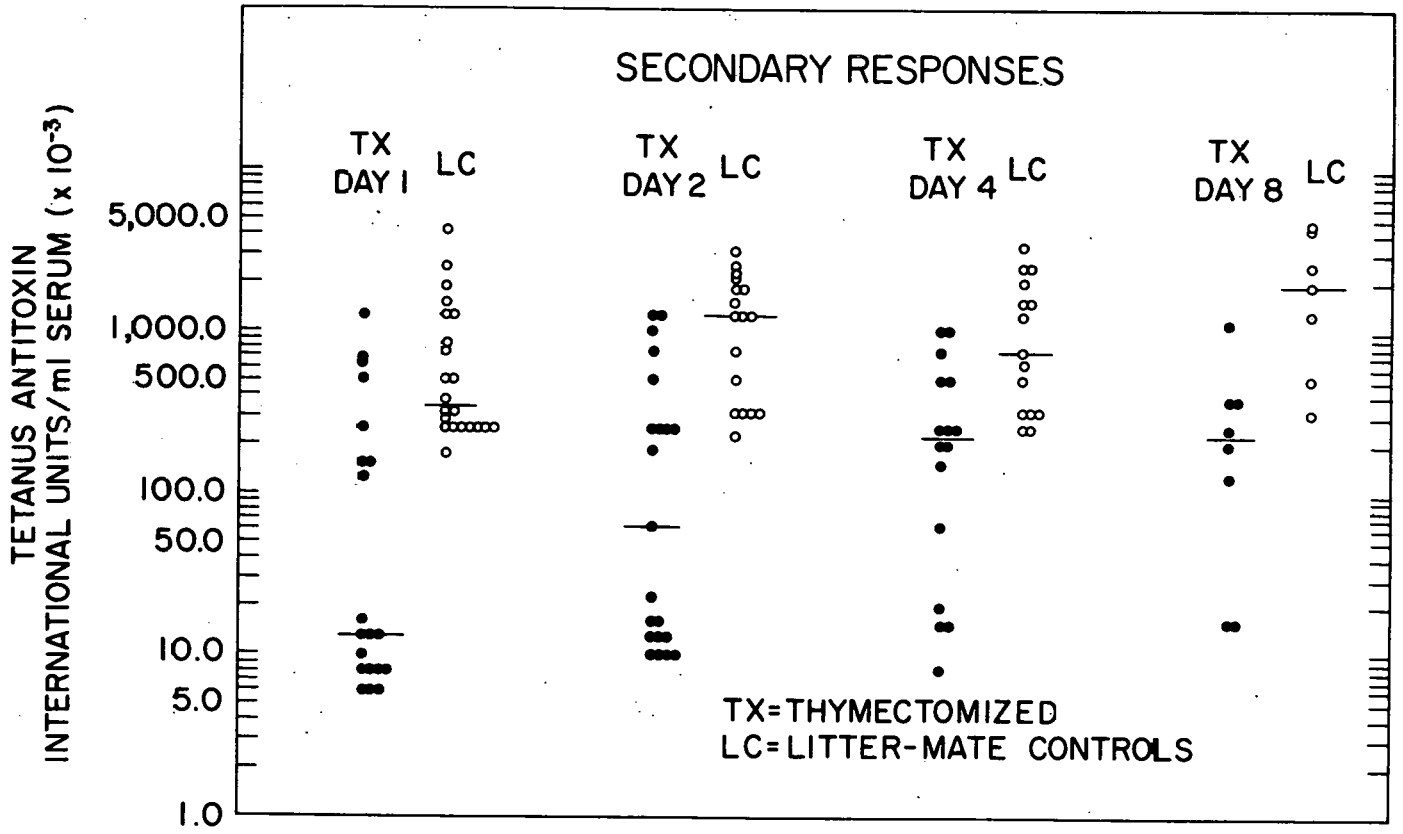
SECONDARY RESPONSES
TETANUS ANTITOXIN
INTERNATIONAL UNITS/ml SERUM (x 10⁻³)
5,000.0
1,000.0
500.0
100.0
50.0
10.0
5.0
1.0
TX
DAY 1
LC
TX
DAY 2
LC
TX
DAY 4
LC
TX
DAY 8
LC
TX=THYMECTOMIZED
LC=LITTER-MATE CONTROLS

FIGURE 2

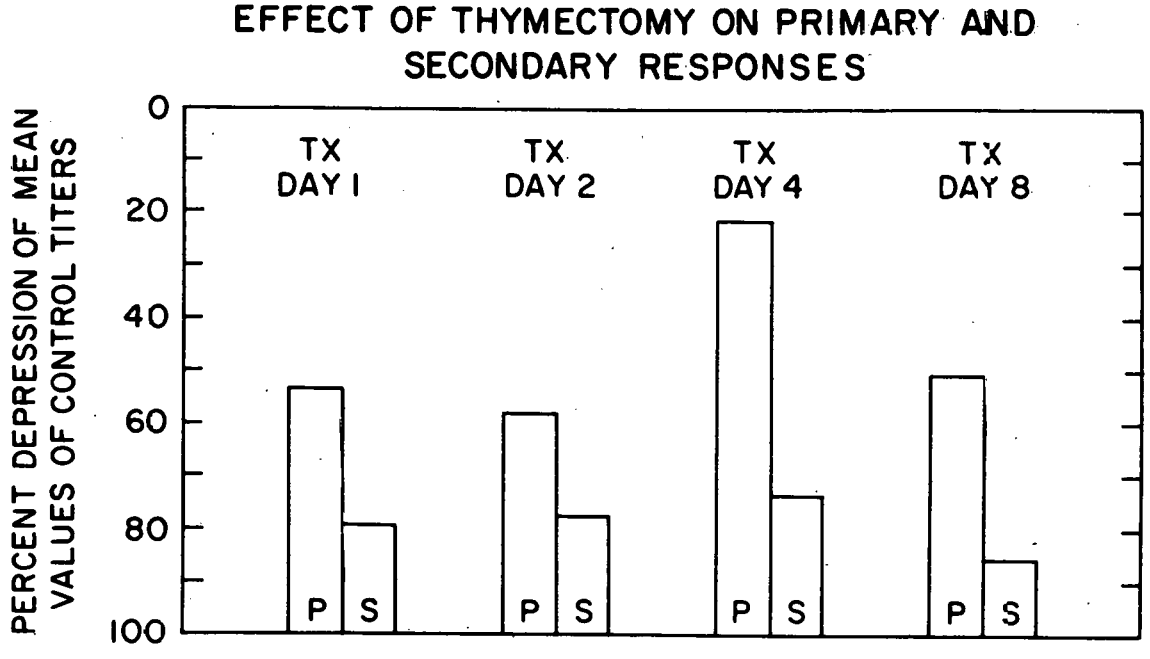
EFFECT OF THYMECTOMY ON PRIMARY AND SECONDARY RESPONSES
PERCENT DEPRESSION OF MEAN VALUES OF CONTROL TITERS
0
20
40
60
80
100
TX DAY 1
TX DAY 2
TX DAY 4
TX DAY 8
P
S
P
S
P
S
P
S

FIGURE 3

TABLE 1

THE EFFECT OF THYMECTOMY ON BODY WEIGHT IN MICE

AGE	TX - Day 1		LC		TX - Day 2		LC		TX - Day 4		LC		TX - Day 8		LC	
	n	g	n	g	n	g	n	g	n	g	n	g	n	g	n	g
28 days	22	13.7 ± 0.9	22	14.9 ± 0.7	21	14.3 ± 0.9	18	14.8 ± 1.0	18	15.3 ± 1.0	15	15.8 ± 1.2	8	13.8 ± 2.8	7	15.1 ± 2.6
49 days	22	18.3 ± 1.8	22	22.0 ± 0.9	21	21.3 ± 1.5	18	21.6 ± 1.0	17	22.2 ± 1.7	15	23.0 ± 1.4	8	21.3 ± 1.9	7	21.5 ± 1.2
59 days	22	19.8 ± 5.0	11	23.2 ± 0.1	20	23.1 ± 1.7	11	23.0 ± 1.7	16	23.8 ± 2.1	11	22.7 ± 2.2	8	21.9 ± 4.5	7	22.3 ± 2.7

TX = thymectomized
LC = litter-mate controls
n = number of animals
g = body weight in grams