Growth and Regression of the Germfree (Axenic) Thymus.*† (29766)

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(Introduced by Morris Pollard)
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Attention is currently focused on the thymus as having a key role, either directly by a cellular factor or indirectly by a humoral factor, in the ontogeny of immunity and the development of the peripheral lymphoid tissues in mammals and other vertebrates(1). Much information has been accumulated concerning the crippling of the immune response by thymectomy and the subsequent development of wasting disease in the neonatally thymectomized animal(2). Thymectomized germfree (axenic) mice, however, have not developed this wasting syndrome(3).

Although germfree animals usually show a lower development of lymphoid tissue and lower serum β- and γ-globulin levels, they are capable of responding well to antigenic stimuli(4). According to Bauer and associates, the underdeveloped immunologic system of the germfree animal is capable of an equal though slightly delayed response to bacterial antigens(5). This underdevelopment may be the result of minimal antigenic stimulation, and represents a quantitative rather than a qualitative difference between germfree and conventional animals(6). A correlation between immunological activity and development of the lymphoid tissues was sought in a comparison of germfree and conventional mice. This study was undertaken to determine whether the growth activity of the thymus of germfree mice, devoid of appreciable antigenic stimulation, differs significantly from that of the conventional mice, which are continually the subject of antigenic stimulation.

Materials and methods. In this study 198 germfree and 133 conventional mice of the Lobund-maintained CFW strain were autopsied. The thymus weights are reported here for a comparison of the growth activity and regression of the thymus. Germfree animals were studied to 457 days and the conventional to 315 days.

The germfree mice were housed in plastic cages in Trexler flexible film isolators(7), and maintained by the routine procedures developed at the Lobund Laboratory for germfree animals(8,9). Animals were fed *ad libitum* on a diet of sterile Purina Lab Chow, 5010 C. Although these animals are not claimed to be virus- and *Rickettsia*-free, conventional examinations for such organisms have to date given negative results with the exception of leukemia virus demonstrated by Pollard and associates(10,11). Conventional mice were derived from germfree mice which had been adapted to the microflora of the animal quarters and were housed in plastic cages on the laboratory shelf. There was no significant difference between the weights of the germfree and conventional mice of the same age.

Results. A comparison of the growth activity and regression of the thymus of germfree and conventional CFW mice is shown in Fig. 1. Values are expressed as the ratio of thymus weight to body weight. During the period of accelerated growth the conventional thymus grows significantly faster than the germfree thymus; the ratio triples in the conventional mouse during the first 24 hours after birth and only doubles in the germfree (Table I). Peak values are reached at 15 days in the germfree and at 16 days in the conventional, with the peak value for conventional significantly much higher than germfree statistically. The values of both decrease rapidly after 15-16 days until about

* Supported by funds from Office of Naval Research, Nonr-1623(15).

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TABLE I. Ratio of Thymus Weight to Body Weight in Germfree and Conventional CFW Mice.*

Age (days)	Germfree No.	Germfree Thymus wt (mg)/ body wt (g)	Germfree Ratio $\times 10^{-3}$ ± S.E.	Conventional No.	Conventional Thymus wt (mg)/ body wt (g)	Conventional Ratio $\times 10^{-3}$ ± S.E.
0	8	.99/ 1.25	.81 ± .1	4	1.55/ 1.37	1.12 ± .3
1	6	3.00/ 1.49	2.06 ± .2	8	5.75/ 1.50	3.70 ± .5
4	2	3.50/ 2.20	1.58 ± .1	4	12.50/ 2.90	4.31 ± .1
7	4	24.25/ 4.10	2.44 ± 1.03	5	25.20/ 6.01	4.04 ± .6
14	7	43.29/11.78	4.58 ± .8	3	55.33/ 8.13	6.82 ± .5
15	6	45.85/ 6.43	7.11 ± .3			
16	4	52.00/ 7.52	6.90 ± .5	4	89.00/10.00	8.90 ± .2
19	6	30.00/ 6.93	4.98 ± .3	3	36.67/ 5.93	6.10 ± .3
49	2	45.00/21.29	.21 ± .0	2	96.50/22.40	4.35 ± .1
315				2	15.50/30.40	.51 ± .1
457	1	12.00/30.00	.40 ± .0			

* Table I is an abbreviation showing representative values taken from the table deposited with the American Documentation Institute (ADI), Library of Congress. Fig. 1 was constructed from the unabridged table. Photostat or microfilm copies are available at moderate cost. Values for 0, 1, 4, and 7 days of age represent the period of accelerated growth; 14, 15, and 16 days of age represent the peak values; 19 days is found in the period of decelerated growth; around 49 days of age the period of regression begins; and 315 and 457 days of age represent values from the oldest animals studied.

50 days. They reach a plateau near 100 days with values within the range of those found in the newborn. The thymus never completely disappears. There is no statistically significant difference in the curves from 20 days to the end of the period of observation, although the ratio in conventional animals always remains slightly larger than in the germfree. The thymus actually reaches its maximum size at 50 days. The decrease in ratio of thymus weight to body weight between 16 and 50 days probably reflects a combination of more rapid increase in body weight and deceleration of thymus growth rate. Actual regression of the thymus begins at 50 days and reaches a plateau around 200 days.

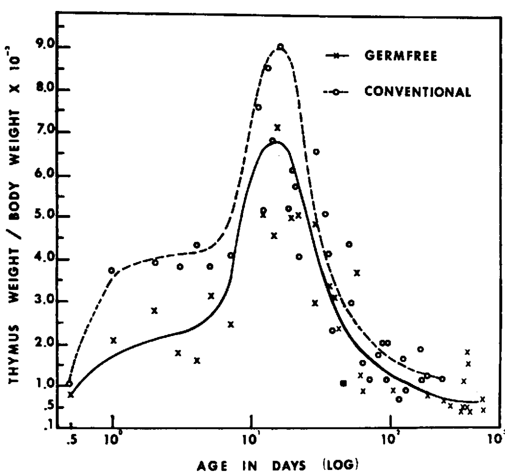

FIG. 1. Ratio of thymus weight to body weight in germfree and conventional CFW mice from birth to 457 days of age.

Discussion. If the development of the lymphoid organs of an animal is dependent upon the thymus, a difference in thymus activity would be expected to alter such development. The thymus growth patterns of germfree and of conventional mice are consistent with the differences in morphology and function of lymphoid tissues effected by the germfree state as reported by others(4,5). The thymus is believed to exert its chief influence during the early life of the animal. The thymus of the conventional animal is much more prolific during the first week after birth than in the germfree animal and ultimately it achieves a greater size. There is no difference in the gross appearance of the thymus in germfree and conventional mice. The size of the thymus is dependent principally upon the size of its thymocyte population. The germfree animal is spared from the exposure to environmental microorganisms that the conventional animal experiences after birth and consequently, from the antigenic stimulation resulting from such exposure. This probably explains the difference observed in the thymus growth patterns. Germfree animals, however, are not entirely without antigenic stimuli. These emanate primarily from the diet which may contain antigens from killed bacteria as well as certain plant and animal antigens, and possibly occult viruses which are doubtlessly disseminated by congenital routes.

Our data correlate very well with those of Ball(12), who found that the number of small lymphoid cells in the thymus increased by a factor of 10 between birth and 14 days post-partum and that the maximum number of cells was achieved around 50 days of age. The embryonic rate of increase, however, is substantially greater than that after birth; embryonic growth was not a part of this study.

A number of factors cause variation in size of the thymus: stress conditions such as ether anesthesia, exposure to cold or extreme heat, lack of food, and crowded cage conditions may cause shrinkage of the organ. Pregnant females beyond the age at which regression normally occurs have enlarged thymuses. The time that elapsed between removal of germfree animals from the isolators and autopsy also contributed to variation in thymus size.

Summary. Qualitatively, the growth patterns of the thymus of germfree and conventional mice are very similar, and the gross appearance is the same. In both groups of animals the period of thymus growth extends from birth to about 50 days, with accelerated growth during the first 2 weeks after birth, and peaks at about 15 days. Growth continues at a decelerated rate and regression of the thymus begins at about 50 days of age. A plateau is reached at about 200 days of age, but the thymus does not disappear completely. However, the thymus of conventional mice grows more rapidly and attains greater size than that of the germfree mice.

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Received September 2, 1964. P.S.E.B.M., 1965, v118.

Direct Luteinizing Activity of FSH-NIH.* (29767)

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FSH-NIH-S1 and S2 have luteinizing potencies, in relation to LH-NIH-B1, of 0.006(1) and 0.00028†, respectively, by the ovarian ascorbic acid depletion (OAAD) test(2). LH activity may also be determined by other methods, including that of production of corpora lutea in ovarian isografts in male mice(3). The present report concerns this type of response to the 2 FSH preparations.

Materials and methods. Groups of 8-15 male mice (BALB/c strain) 3-6 months of age, received bilateral intraocular ovarian isografts from females of the same age. Two to 4 weeks later, each animal in a group received a single specific dose of FSH-NIH-

* Supported by USPHS Research Grant CA-02880.

† FSH-NIH-S1 and S2 and LH-NIH-B1 were generously supplied by the Endocrinology Study Section, NIH. In the absence of a specific reference in the text, potency estimates were taken from the protocols accompanying these gonadotropins.