The Absence of Wasting in Thymectomized Germfree (Axenic) Mice.* (29545)

RAPHAEL WILSON, KENNETH SJODIN† AND MARGARET BEALMEAR
(Introduced by Morris Pollard)
Lobund Laboratory, Department of Biology, University of Notre Dame, Notre Dame, Ind.

Neonatal thymectomy in mice results in marked impairment of the immune response to standard antigens and skin homografts, as well as severe depletion of the lymphocyte population of the blood and lymphoid tissues (1).

A disease commonly designated as wasting disease develops in animals thymectomized within 24 hours after birth. This condition is characterized by loss of weight or failure to gain weight at a normal rate, cervical alopecia, ataxia, kyphosis, lethargy, ruffled fur, edema around the face and eyes, diarrhea, lymphocytopenia, and lymphoid tissue atrophy(2). The syndrome appears from 4 weeks to 4 months after thymectomy depending on the strains of animals used(1). The wasting disease following neonatal thymectomy has obvious resemblances to the syndromes seen in graft-*vs*-host conditions, such as runt disease in infant mice, secondary disease in radiation chimeras, F_1 hybrid disease, and parabiotic intoxication(3).

Experimental evidence indicates that the thymus of adult mice does not play an appreciable role in formation of plasma cells or their precursors nor does it participate in gamma globulin synthesis. However, it does appear to be an important source of circulating lymphocytes or of a factor which stimulates lymphocytosis(4). According to Miller, the recovery of lymphopoietic and immunogenic functions in an irradiated *adult* mouse is still dependent on a thymus-controlled mechanism(1).

Up to the present, studies of the wasting disease syndrome have met with many technical complications. Animals die before extensive investigations of their immunological responsiveness can be made. The unanswered question is whether the wasting syndrome is simply a secondary infection resulting from the absence of an immune response on the part of the host, or whether it is due to some other factor resulting from thymectomy. Bacterial cultures of heart blood and surgical sites in wasted animals have been uniformly negative. Fecal cultures showed the same microbial flora as non-operated animals(2). Parrott and East have found evidence of an hepatotropic virus, probably mouse hepatitis virus-1 (MHV-1), in the liver of about 50% of mice dying of wasting disease(5). Since the germfree status rules out infection, we have employed germfree CFW and C3H mice to study the wasting syndrome, to determine whether the disease results from failure of the immune response to antigenic stimuli or of some other factor.

Materials and methods. In these studies 40 C3H and 118 CFW axenic and 19 C3H and 263 CFW conventional mice of the Lobund-maintained strains were thymectomized within 24 hours after birth; 26 CFW axenic mice were thymectomized at 6 days, and 7 CFW axenic mice at 44-51 days of age. The axenic mice were housed in plastic cages in Trexler flexible film isolators(6), and maintained by the routine procedures developed at the Lobund Laboratory for axenic animals(7,8). Animals were fed *ad libitum* on a diet of sterile Purina Lab Chow, 5010 C. By definition, the axenic animal is free of living bacterial, mycologic, protozoan, and macroparasitic associates within the limits of our routine microbiological examination (9). Although these animals are not claimed to be virus- and *Rickettsia*-free, examinations for such organisms to date have been negative 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. Conven-

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

† Dept. of Physiology, Univ. of Minnesota, Minneapolis.

tional mice were housed in plastic cages on the laboratory shelf.

The technique used in neonatal thymectomy of mice was a modification of the technique described by Sjodin *et al* (12). Maintenance of the germfree status required modifications of the technique because of the physical conditions within a germfree isolator. An operating chamber with an optical plastic window over which a dissecting microscope could be placed, was added to a Trexler-Type flexible film isolator. Surgical gloves were attached to the sleeves of the operating compartment. All surgical procedures are carried out under germfree conditions. Hypothermic anesthesia was administered by means of an anesthetizing chamber extending through the wall of the isolator into a cold box outside. A vacuum pump was attached to the isolator through a glass tube sealed into the isolator wall. Plastic operating boards were designed which could be sterilized with peracetic acid(13).

An incision was made on the chest through the skin and sternum down to the fourth rib with microdissection scissors; the thymus was visualized and removed by gentle suction. Wounds were closed with black braided silk ophthalmic sutures, No. 6-0 with 3/8 circle taper needle. After cleansing the skin with sterile hemosol solution, plastic wound dressing was applied over the incision with a dental probe. When fully recovered the animals were returned to their mothers. The same technique was followed for thymectomy of conventional mice with the exception that no germfree isolator was needed.

Results. Over-all results are summarized in Table I. With the technique used, mortality due to surgery in axenic mice was 8.4% and in conventional mice 12.1%. Deaths due to cannibalism were about 59% for axenic and 54% for conventional mice.

Nine CFW mice were maintained in the germfree state for 4 months after neonatal thymectomy—the longest period for this strain, and no symptoms of wasting disease developed (Table II). The animals developed and reproduced normally. Their conventional counterparts died from the wasting syndrome at 4-8 weeks. After 4 months the isolator became accidentally contaminated and the animals were conventionalized. One of the animals was dead within 2 weeks with signs of severe wasting disease; the 8 remaining died within 2 months. Seven CFW germfree mice thymectomized at 44-51 days became accidentally contaminated at 147 days. They were sacrificed and autopsied at 215 days; blood counts, body weights, lymphoid organs, etc., appeared to be normal. The remaining contaminated mice are specified in Table II.

TABLE I. Summary of Results.

	Axenic		Conventional	
	C3H	CFW	C3H	CFW
Thymectomized	40	151	19	263
Lost in surgery	5	11	3	31
% Lost in surgery	8.4%		12.1%	
Lost by cannibalism	8	92	10	123
% Lost by cannibalism	59.5%		53.6%	
Lost by contamination	10	21		
Sacrificed	9	11	0	22
Surviving (# surviving/# thymectomized)	4/4	20/20	0/6	5/87
Dead @ 4 wk from wasting	0/4	0/20	6/6	44/87
Dead @ 6-8 wk from wasting	0/4	0/20		38/87

At present 4 C3H mice have been maintained in the germfree state for 8 months after neonatal thymectomy without developing any signs of wasting disease (Table III). Five of their thymectomized litter mates have been sacrificed at intervals and histological sections made to check for complete thymectomy. All have been found to be thymus-free. Thirteen CFW mice thymectomized at birth and 7 at 6 days are being maintained germfree at present, and the first 6 of these (thymectomized at birth) show no visible signs of wasting 4 months after thymectomy (Table III).

Discussion. Without the use of germfree animals it would be difficult to determine the mechanism by which the thymus performs its function. Germfree mice, thymectomized at birth, do not develop the characteristic wasting syndrome, yet upon contamination and subsequent conventionalization, they succumb to wasting disease in the same length

TABLE II. Development of Wasting Syndrome in Ex-Germfree Thymectomized Mice.

Date of thymectomy	Strain of mice	No.	Age (days) at Thymectomy	Age (days) at Contamination	Time of death after conventionalization
2/ 1/63	CFW	7	44–51	147	Sacrificed @ 215 days
8/16/63	CFW	9	0	120	1 @ 2 wk, remainder @ 4-8 wk
10/ 1/63	C3H	5	0	24	4-8 wk
10/14/63	C3H	3	0	11	4-8 "
10/21/63	C3H	2	0	4	4-8 "
11/14/63	CFW	5	0	30	4-8 "

TABLE III. Surviving Axenic Thymectomized Mice.

Date of thymectomy	Strain of mice	No.	Age at thymectomy (days)	Comments
9/ 9/63	C3H	4	0	Still alive and reproducing normally @ 231 days
12/26/63	CFW	6	0	Still alive and reproducing normally @ 113 days
2/19/64	CFW	7	6	Still alive and reproducing normally @ 68 days
4/21/64	CFW	7	0	Still alive and normal

of time that it normally takes a conventional neonatally thymectomized animal to succumb. Germfree mice thymectomized at 44-51 days develop none of the wasting disease symptoms when contaminated and later conventionalized. The absence of pathogenic bacteria in the diseased animals suggests that perhaps a virus, *Rickettsia,* or a fastidious bacterium may be responsible for the disturbance. If so, germfree technology, by permitting the introduction of specific species of microorganisms into the system should provide a method for identification of organisms responsible for the wasting syndrome. Of much significance in this report is the evidence that the complications of post-thymectomy infections can be avoided during the course of investigation, thereby providing a long period of axenic study and consequently reducing variability resulting from otherwise associated microorganisms.

Summary. Neonatally thymectomized mice develop a characteristic wasting disease from 4 weeks to 4 months after thymectomy; no pathogenic bacteria have been isolated from these animals that can be obviously incriminated as the cause of the syndrome. Germfree thymectomized mice exhibit none of the symptoms of this disease in 8 months, the maximum period of observation to date. Upon contamination the ex-germfree mice, thymectomized at birth, develop the disease within 4-8 weeks, though those thymectomized at 44-51 days do not develop the disease upon contamination and subsequent conventionalization.

1. Miller, J. F. A. P., *Brit. Med. J.,* 1963, v2, 459.
2. Sherman, J. D., Adner, M. M., Dameshek, W., *Blood,* 1963, v22, 252.
3. Schlesinger, M., Mark, R., *Science,* 1964, v143, 965.
4. Metcalf, D., *Ann. N. Y. Acad. Sci.,* 1958, v73, 113.
5. East, J., Parrott, D. M. V., Chesterman, F. C., Pomerance, A., *J. Exp. Med.,* 1963, v118, 1069.
6. Trexler, P. C., Reynolds, L. I., *Appl. Microbiol.,* 1957, v5, 406.
7. Reyniers, J. A., *Ann. N. Y. Acad. Sci.,* 1959, v78, 3.
8. Trexler, P. C., *Bio-Medical Purview,* 1961, v1, 47.
9. Wagner, M., *Ann. N. Y. Acad. Sci.,* 1959, v78, 89.
10. Pollard, M., Salomon, J. C., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 256.
11. Pollard, M., Matsuzawa, T., *ibid.,* 1964, in press.
12. Sjodin, K., Dalmasso, A. P., Smith, J. M., Martinez, C., *Transplantation,* 1963, v1, 521.
13. Wilson, R., Sjodin, K., Bealmear, M., Hakes, R., 1964, in preparation.

Received May 4, 1964. P.S.E.B.M., 1964, v117.