of either species was the same (Table 1). Ants and the roach could better detect the odor-trail substance from major than from minor workers of *Atta texana*, evidently because sacs of major workers contained more pheromone.

Roach nymphs of ages 3 weeks, 3 months, and 11 months (growth to sexual maturity takes about 12 months) followed artificial trails as well as did mature females. Five roaches that did not respond to the substance in laboratory tests were *Periplaneta americana* (Linn.), *P. fuliginosa* (Serv.), *Blattella germanica* (Linn.), *Supella supellectilium* (Serv.), and *Parcoblotta* sp.

In the laboratory, the antennae of *Attaphila* moved vigorously but never touched the artificial trail. However, the maxillary palps, which are almost as long as the antennae, were in constant contact with the trail.

The roach has not been found in field nests of *Trachymyrmex*, which are abundant and often superimposed on the larger nests of *Atta*. In the laboratory, roaches survive well in nests of both ants but workers of *Trachymyrmex* are somewhat hostile.

Attaphila fungicola has never been observed on field trails of the town ant, though Bolivar (*2*) recorded *Attaphila schuppi* Wasm. on trails with workers of *Acromyrmex* prob. *niger* (F. Smith) in Brazil. Some individuals of *A. fungicola* placed on field trails followed scent but took about 15 minutes to become adjusted.

Although the roach may not use field trails as a mechanism of dispersal, the pheromone may assure continued association of the insects. It may explain why roaches are often found riding on town ant queens during mating flights.

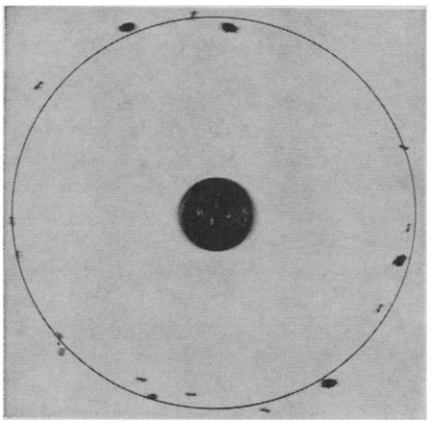

Fig 1. First-instar nymphs and female adults of roach, and minor workers of town ant following artificial trail. Note that insects travel in both directions.

JOHN C. MOSER
Southern Forest Experiment Station, Forest Service, Alexandria, Louisiana

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Humoral Thymic Factor in Mice: Further Evidence

Abstract. *Mice of the C3H and DBA strains thymectomized at birth showed a consistent and striking suppression of antibody production (hemolysin response) to sheep erythrocytes. When cell-tight Millipore diffusion chambers containing syngeneic thymic tissue were implanted intraperitoneally, the capacity of these neonatally thymectomized mice to respond to this antigen was restored. The pattern of response and the mean titer were similar to the pattern and mean titer observed in neonatally thymectomized mice bearing subcutaneous grafts of syngeneic thymic tissue. These data are consistent with the concept that thymic tissue within the chamber produced a specific diffusible factor that enabled the thymectomized animal to establish immunologic competence.*

Thymectomy in mice performed within 24 hours of birth usually leads to a syndrome characterized by a gradual deterioration in physical condition evidenced by progressive weight loss, cachexia and lethargy, ruffled fur, diarrhea, and—terminally—a severe depletion of small lymphocytes in lymphoid organs and blood (*1–3*). The onset and extent of "wasting" and depletion varies with the strain of mouse.

Impaired immunological competence has also been observed. This has been demonstrated by skin homografts (*1, 4*), by grafts of foreign cells (*2, 5, 6*), both normal and neoplastic, and by primary stimulation with several antigens, notably sheep erythrocytes, *Salmonella H* antigen, killed influenza virus, and bovine serum albumin (*5, 7, 8*).

The thymus is an active site of lymphopoiesis in the newborn mouse and it is probably wholly responsible for the production and delivery of immunologically competent lymphocytes early in life.

One important role of the thymus in establishing and maintaining the integrity of the lymphoid system is mediated through a diffusible factor. Neonatally thymectomized C3H/Lw mice implanted with cell-tight Millipore diffusion chambers containing syngeneic (isologous) thymic tissue did not show depletion of lymphocytes in the blood, involution of lymphoid organs, or characteristic signs of the "wasting" syndrome (*3*). Also, neonatally thymectomized mice, bearing diffusion chambers with thymic tissue, regained the capacity to reject skin homografts (*9*). Further confirmation of the action of a humoral mechanism of the thymus was obtained through studies of the response of NIH Swiss mice to lymphocytic choriomeningitis (LCM) virus. Thymectomized mice were protected from the lethal effects of virus; those implanted with cell-tight Millipore diffusion chambers containing newborn thymic tissue had their susceptibility restored to the lethality of LCM infection (*10*).

This study was undertaken to obtain further evidence for a diffusible factor or factors in the thymus. Since neonatally thymectomized mice showed a regular and pronounced suppression of sheep erythrocyte hemolysin response, antibody production (hemolysin response) of neonatally thymectomized mice was compared with the response in neonatally thymectomized littermates in which a Millipore diffusion chamber containing syngeneic thymus was implanted intraperitoneally or in which syngeneic thymus tissue was implanted subcutaneously.

Two inbred strains of mice were used, C3Hf/Lw and DBAf/2Lw. Newborn animals were thymectomized under ether anesthesia within 12 to 18 hours of birth by a technique adapted from Miller (*11*). Two mice in each litter served as controls; these were either sham-operated or intact. Sham operations were included in the first of three groups of C3H mice but were discontinued when no differences were found between these and intact mice.

Animals were killed and autopsied at 6 to 10 weeks of age. Completeness of thymectomy was checked by inspection, and serial sections of suspected thymic tissue in the anterior mediastinum were made. Those few mice with thymic remnants will be discussed specifically.

At the age of 3 to 4 weeks, thymectomized mice were divided into three groups: (i) animals given no further treatment (thymectomized controls); (ii) animals grafted subcutaneously with syngeneic thymus, either one lobe or one entire thymus from 3- to 8-day-old donors (C3H or DBA); and (iii) animals given an intraperitoneal implant of a cell-tight Millipore diffusion chamber containing one complete thymus or one lobe, depending upon the age of the donor. The donor mice, those contributing syngeneic thymuses, were 3 to 8 days old; they were of the C3H or DBA strains.

The Millipore diffusion chambers were constructed by modification of the Algire method (*12*). A Lucite ring with an outside diameter of 10 mm, an inside diameter of 6.4 mm, and a depth of 1.6 mm was used. A Millipore disk (0.45 μ pore size and 150 μ thick) (*12*) was glued to each surface. This pore size prevents passage of cells through the membrane wall (*13*). At the time of necropsy there was no gross evidence of disruption of the integrity of the chamber in any of the mice. The rings and disks were sterilized before use.

Washed, fresh sheep erythrocytes, 0.1 ml of a 10-percent suspension, were injected intraperitoneally 7 to 10 days after implantation of the chamber or after thymic grafting. Mice were bled from the retro-orbital sinus 7 days later, and the serum was titrated for hemolysins by a modification of methods described (*14*). Lyophilyzed guinea pig complement was titrated against a standard antiserum. Titrations were performed with a microtiter system. Serial twofold dilutions of a 1:5 dilution of mouse serum was used. End points corresponded to an antiserum dilution at which approximately 25 percent of the added cells sedimented and approximately 75 percent had hemolyzed. Titrations were also made in the presence of 0.1*M* mercaptoethanol. No hemolysin activity persisted in either normal or thymectomized mouse serum after treatment with 0.1*M* mercaptoethanol. The hemolysin is predominantly of the high molecular weight (18*S*) type (*14*) of antibody.

Typical changes already reported (*10*) in total-body weight and in lymphocyte depletion of blood and lymphoid organs were observed in both C3H and DBA thymectomized mice. Deterioration began at 4 to 5 weeks in C3H mice and 1 to 2 weeks later in DBA mice.

The production of antibody hemolysins to sheep red blood cells is shown for the several groups of C3H mice in Fig. 1 and for a smaller sample of DBA mice in Table 1. Three separate experiments with C3H mice were combined because the results were comparable.

Table 1. Production of sheep RBC hemolysins in C3H and DBA mice.

Strain	Mean peak titers* (range)	Animals responding: No. at 20 and more	Animals responding: Combined (%)
Intact or sham-operated			
C3H	205 (20–640)	46/48	96
DBA	310 (160–640)	8/8	
Thymectomy + (syngeneic thymic grafts)			
C3H	149 (20–640)	11/11	95
DBA	55 (10–80)	8/9	
Thymectomy + (diffusion chamber with thymic tissue)			
C3H	87 (0–320)	29/40	61
DBA	30 (0–160)	5/11	
Thymectomy			
C3H	8 (0–40)	7/24	24
DBA†	3 (0–20)	1/9	

* Reciprocal of titer. † These mice were implanted with empty Millipore diffusion chambers.

The mean of the highest hemolysin titer for 48 intact C3H mice in this group was 205 and for their 25 thymectomized littermates the mean was only 8. This consistent and striking depression of immunologic capacity in C3H mice was seen in preliminary observation in which the mean of the highest hemolysin titer of 29 neonatally thymectomized mice was 6.5 (0 to 40) compared with a mean titer of 335 (120 to 640) in 21 littermate controls composed of intact or sham-operated animals.

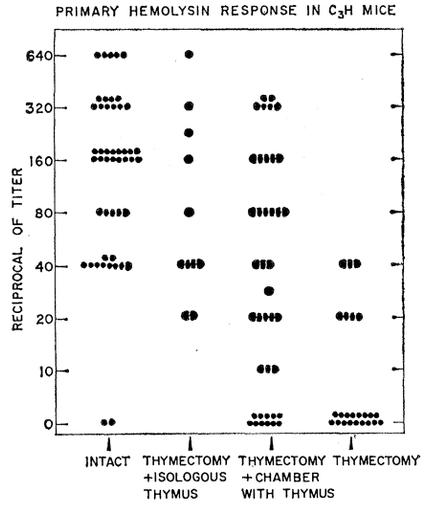

Fig. 1. Comparative hemolysin production determined at day 7 among littermates of C3Hf/Lw mice. Isologous (syngeneic) thymic grafts are C3H and were grafted in the subcutaneous connective tissues of the right axillary region. Millipore diffusion chambers contained syngeneic C3H thymuses and were implanted intraperitoneally. Only completely thymectomized mice, as checked at necropsy, are included. See Table 1 for summary of results.

Seventeen out of 24 (71 percent) C3H thymectomized mice in the present group failed to give titers detectable by our methods.

Twenty-six out of 40 (65 percent) thymectomized mice bearing the cell-tight Millipore diffusion chambers containing thymic tissue gave an antibody response pattern (at 1:20 and above) and a mean titer of response (mean = 133) similar to their C3H littermates grafted with isologous thymic tissue (mean = 149). Both mean titers however were lower than that obtained in intact littermates (mean = 205).

The restorative action of thymic tissue in the Millipore chamber may be seen by comparing the thymectomized mice that formed antibody at titers of 1:20 and above (29.2 percent) with their littermates implanted with chambers containing thymus (65 percent). The difference, 35.8 ± 12.8 percent is significant with $P < 0.01$.

In the C3H mice with implanted chambers, a positive correlation was not always found between immunologic responsiveness and other criteria of restoration. Some mice that showed high hemolysin titers nevertheless were "wasted" and continued a downhill course; others showed depletion of lymphoid tissue or disturbance of the lymphocyte-granulocyte ratios. In some animals that showed antibody response necrotic tissue was found within the diffusion chamber at necropsy, usually at 40 to 60 days of age.

Thymic remnants were observed in four C3H mice, two from the thymectomized group and two from the thymectomized group implanted with thymus-containing Millipore chambers. The remnants measured, on the average, 1 by 1 by 1 mm. In all these mice there was a steady gain in body weights, or normal peripheral blood lymphocyte population and hemolysin titers, of 20, 80, 80, and 320, respectively. These are not included in the results of Fig. 1.

The results of hemolysin production in DBA mice are shown in Table 1, along with a summary of the results in C3H mice. Although preliminary, these data show the same dramatic immunologic deficiency among the thymectomized mice and apparent partial restoration of this deficiency in those animals bearing thymus-containing Millipore diffusion chambers or syngeneic thymic

grafts. Here, all of the control thymectomized DBA mice had been given intraperitoneal implants of empty diffusion chambers. This type of response would appear to rule out an adjuvant effect of the Millipore material (*10*).

The viability of the thymic tissue in the chamber appears finite (*3*). In many of the chamber-bearing mice that produced antibody, necrotic tissue was found at necropsy, indicating an early beneficial effect similar to that reported in bursectomized chickens bearing bursal implants. In this case there was an enhanced formation of agglutinating antibody to *S. typhimurium* although bursal homografts were uniformly rejected by 8 days (*15*).

The diminished immunologic capacity after neonatal thymectomy, shown by depression of antibody production to most antigens, by depression of delayed hypersensitivity-like reactions, and by inability to reject homografts, has been noted also in the rat and hamster (*16*).

Subcutaneous thymic grafting in the mouse with syngeneic thymic tissue during the first week of life (*8*) or as late as 3 to 4 weeks (*17*) effectively prevented the deficiencies of thymectomy. Cytologic studies with chromosome markers (*18*) showed that the majority of cells multiplying within the thymic graft and in lymphoid organs were of host type and not of thymic graft origin. These results strongly suggested the existence of a factor, noncellular in nature, that contributes to the establishment of immunologic competence, either within the environment of thymic tissue itself or through its elaboration and effect upon seeded cells within lymphoid organs. The results presented here suggest the elaboration of a diffusible thymic factor that acts upon seeded cells in lymphoid organs, but they do not rule out the alternative concept.

L. W. LAW, N. TRAININ*
R. H. LEVEY,† W. F. BARTH
National Cancer Institute,
Bethesda 14, Maryland

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* Permanent address: Department of Experimental Biology, Weizmann Institute of Science, Rehovoth, Israel.
† Present address: Department of Surgery, Massachusetts General Hospital, Boston.

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Neuraminidases and Influenza Virus Infection in Embryonated Eggs

Abstract. *Highly purified neuraminidase from influenza virus prevents embryonated eggs from infection by Lee-B influenza virus. The degree of prevention afforded is equal to that of neuraminidases from* Vibrio cholerae *and from* Clostridium perfringens *when assayed at the same enzyme activity. The preventive effect is much lower against PR-8 virus. The results support the concept that the intact "receptor" containing sialic acid is required for the binding and infection of cells by influenza viruses.*

Stone (*1*) first showed that neuraminidase preparations from *Vibrio cholerae* (RDE) could prevent infection of embryonated eggs by influenza virus. She also showed that intranasal instillations of this enzyme preparation protected mice against lung infection by viruses of the influenza group (*2*).

Fazekas de St. Groth also demonstrated the enzymatic destruction and regeneration of influenza virus receptor groups (*3*). These observations have been interpreted to be the result of the destruction of specific cellular receptor sites by the enzyme, thus preventing adsorption of the virus.

This report concerns the similar behavior of a highly purified neuraminidase isolated from influenza virus and demonstrates that, per unit of enzyme activity, bacterial and viral neuraminidases have the same protective effect.

Neuraminidase with specific activities of about 2.5 units (*4*) per milligram of protein was isolated from Asian influenza virus by the methods of Mayron *et al.* (*5*) as modified by Wilson and Rafelson (*6*). Crystalline neuraminidase from Asian virus has an activity of 5.1 units per milligram of protein. Our preparation was isolated from *Vibrio cholerae* essentially by the method of Ada *et al.* (*7*). A preparation with an activity of 0.015 units per milliliter was obtained (*8*). All preparations lacked protease activity when tested by the casein-splitting procedure (*9*).

Neuraminidase with a specific activity of 20 units per milligram of protein was prepared from a culture filtrate of *Clostridium perfringens* by dialysis. The fraction precipitated between 60 and 75 percent saturation with ammonium sulfate was used.

The activity of viral neuraminidase was determined by incubating 0.2 mg of neuraminlactose, prepared by the procedure of Schneir *et al.* (*10*), with 0.4 ml of 0.1*M* phosphate buffer at *p*H 7.0. One-tenth milliliter of the enzyme containing less than 0.005 units was added, and the mixture was incubated at 37°C. Bacterial neuraminidase was assayed in exactly the same way except that 0.1*M* acetate buffer containing 0.01*M* calcium acetate was used. After 30 minutes or 1 hour at 37°C, the reaction was stopped by adding 0.5 ml of ice-cold 5 percent phos-

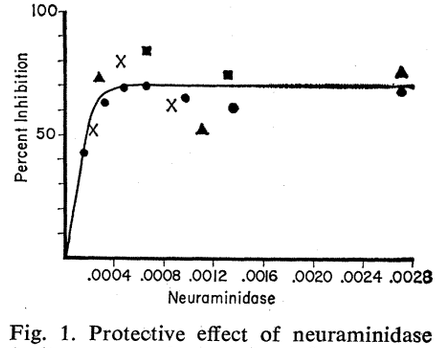

Fig. 1. Protective effect of neuraminidase (units per egg) from Asian influenza virus against Lee-B virus. Results from four separate experiments are shown by the different symbols.