Plasmodium berghei Infection in Thymectomized Rats* (33968)

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Rats that survive a *Plasmodium berghei* infection demonstrate a strong resistance to reinfection when challenged with parasites of the same strain (1). It is assumed that acquired immunity plays some role in controlling the initial infection and in preventing subsequent infections. This conclusion is supported by studies which demonstrate the presence of protective antibody in the serum of previously infected animals (2–6). Enhanced phagocytic activity of the reticuloendothelial system may also be involved in this resistance to reinfection (7–9).

Neonatal thymectomy of the rat is known to alter the animal's immune responses. One of the major effects, that on cellular immunity, is manifested by delayed skin homograft rejection and impaired delayed type hypersensitivity reactions (10, 11). Thymectomized rats also have decreased antibody production following immunization with certain antigens (10, 12, 13).

A preliminary report indicates that mortality from *P. berghei* is higher in thymectomized rats than in nonthymectomized controls (14). No information is available concerning the mechanism by which neonatal thymectomy might alter the immune response to this parasitic infection. The following study confirms the higher mortality from *P. berghei* infection in thymectomized rats and evaluates the antibody response and phagocytic activity in these animals. The susceptibility of thymectomized and nonthymectomized rats to reinfection was also studied.

Materials and Methods. Animals. WRCF rats derived from the Wistar strain were used throughout the study. Litters were weaned at age 20–28 days. All animals were fed a standard diet. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Surgical Procedure. Thymectomy was performed within 36 hr of birth. Hypothermic anesthesia was utilized as previously described (10). Sixty to 70% of the animals in each litter were chosen at random for thymectomy and the remainder were given sham operations. Animals with stitch abscesses or those cachectic in appearance were excluded from the study. All animals that were alive at the end of the experiment were necropsied to verify the results of surgery.

White blood cell counts. In some experiments repeated total WBC and differential counts were made with blood obtained from the tails of the rats when they were 20–30 days old and the mean values for each were determined. The median lymphocyte count of thymectomized animals in any particular experiment was also determined and the rats were then placed in high and low lymphocyte groups.

Infection. The N.Y.U. 2 strain of *Plasmodium berghei* was maintained in the laboratory with biweekly passage in young rats. Groups of experimental animals 28–45 days of age were infected by intraperitoneal injection of parasitized erythrocytes. Progress of the infection was determined by counting parasitized cells per 500 erythrocytes on Giemsa stained smears prepared every 2–3 days from tail bleedings.

Antibody determinations. Serum was obtained by orbital sinus bleedings 11–13 days after infection. Indirect fluorescent antibody titers were determined by methods previously described (15). Indirect hemagglutinating activity of the sera was studied by using tanned sheep red blood cells sensitized with a soluble *P. berghei* antigen (16).

Carbon clearance. The nonspecific phagocytic capacity of the reticuloendothelial sys-

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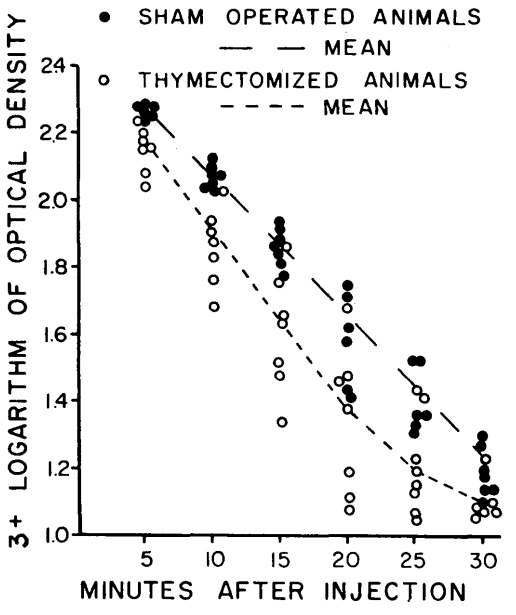

FIG. 1. Disappearance of colloidal carbon from the blood of sham-operated and thymectomized rats following the intravenous injection of 10 mg of carbon/100 mg of body weight.

tem was measured by determining the rate of carbon clearance from blood. Rats were injected intravenously with 10 mg of colloidal carbon[1]/100 mg of body weight; blood specimens were obtained at 5-min intervals and the carbon level and rate of disappearance was calculated (17).

Results. Postoperative mortality. Within 72 hr after surgery 29% of the operated animals had died. This value represents death due to cannibalism, blood loss, and the surgical procedure. By the fourteenth day an additional 6% had succumbed due to undetermined causes and thereafter the mortality rate from causes other than malaria was negligible. The mortality rates of the thymectomized or sham-operated animals were not significantly different.

Effect of thymectomy on carbon clearance. Forty-four-day-old sham-operated and thymectomized animals were studied for rates of carbon clearance (Fig. 1). The average clearance rate in the thymectomized group was slightly faster than that observed in the sham-operated rats.

Effect of thymectomy on the lymphocyte count. Previous workers have shown that thymectomized animals have a decreased population of lymphocytes (11). As shown in Table I, considerable variation in the lymphocyte counts was observed in all groups. Only when the thymectomized group of animals was divided was there a group identifiable by a significant suppression of the number of circulating lymphocytes. At autopsy up to 40% of the animals in some of the high lymphocyte thymectomized groups contained a small remnant of thymic tissue. Thymic remnants were found in less than 5% of the animals in the low lymphocyte groups.

Effect of thymectomy on P. berghei *infection.* In Expt. 1, thymectomized and sham-operated animals at 40 days of age were injected intraperitoneally with 2×10^7 parasitized erythrocytes. The course of the infection is shown in Fig. 2. The level of parasitemia in the high lymphocyte thymectomized rats was the same as that noted in the sham-operated controls. The low lymphocyte thymectomized group had a higher parasitemia for a longer duration and two of the 11 animals died. At age 82 days the groups were reinjected intraperitoneally with 3×10^8 parasitized cells. No parasites were seen in the peripheral blood smears from any of the animals. In repeated experiments using the same conditions, the parasitemia levels of the high lymphocyte thymectomized group

TABLE I. White Blood Cell and Lymphocyte Counts in Normal, Sham-operated and Thymectomized Rats.

Group	WBC (mm^3)	Lymphocytes (mm^3)
Normal (31)[a]	9135 ± 1508[b]	6897 ± 1133
Sham-operated (15)	9783 ± 1933	6945 ± 1401
Thymectomized (10) (high lymphocyte)	9411 ± 2595	5912 ± 1753
Thymectomized (11) (low lymphocyte)	6390 ± 865	3784 ± 1389

[a] Number of animals per group.
[b] Mean ± one standard deviation.

[1] C/11/1431a, Pelikan Special Biological Ink; John Henschel and Company, 425 Park Avenue, New York (U. S. agents for Günther Wagner pelikan Werche, Hannover, Germany).

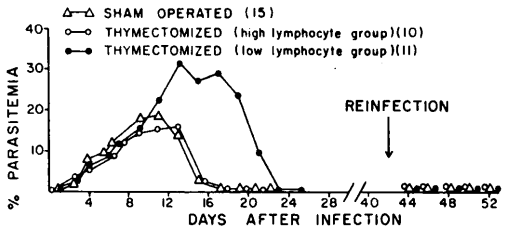

FIG. 2. Expt. 1: Percentage of parasitemia in sham-operated and thymectomized rats infected at 40 days of age with 2×10^7 parasitized erythrocytes.

were intermediate between the sham-operated and low lymphocyte thymectomized groups. The percentage mortality from infection in thymectomized and sham-operated animals is shown in Table II. The mortality in unselected thymectomized rats is, in all experiments, higher than the mortality in sham-operated controls. When the thymectomized animals were divided into a high and low lymphocyte group, the low lymphocyte group had the highest percentage mortality.

Normal animals, comparable to those in Exp. 1 in both age and weight, were injected with the same number of parasites and the course of infection was similar to that

TABLE II. Percentage Mortality in Thymectomized and Sham-operated Rats Infected with *P. berghei*.

Experiment	Infected[a] at age (days)	Mortality (%)	
No. 1	40		
Sham-operated		0	(15)[b]
Thymectomized			
High lymphocyte		0	(10)
Low lymphocyte		18.2	(11)
No. 2	28		
Sham-operated		33	(15)
Thymectomized			
High lymphocyte		50	(10)
Low lymphocyte		100	(10)
No. 3	45		
Sham-operated		0	(15)
Thymectomized[c]		41	(27)

[a] Dose: 2×10^7 parasitized erythrocytes.
[b] Number of animals per group.
[c] No separation according to lymphocyte count was done.

observed in the sham-operated and high lymphocyte thymectomized groups. Five of the normal animals with the highest mean lymphocyte count were compared with the five normal animals having the lowest mean lymphocyte count. The percentages of parasitized RBC in these two groups of normal animals were essentially the same throughout the infection.

Effect of thymectomy on antibody response. The effect of thymectomy on the antibody response to *P. berghei* during the infection was studied by using indirect hemagglutinating and fluorescent antibody techniques. Serum was obtained from 11 to 13 days after infection and the results are shown in Table III. The hemagglutinating titers

TABLE III. Antibody Response to *P. berghei* Infection in Thymectomized and Sham-operated Rats.

Group	Titer	Parasitemia (%)
	Indirect hemagglutinating	
Thymectomized (11)[a]	320[b]	27
Sham-operated (8)	80	12
	Fluorescent antibody	
Thymectomized (11) (low lymphocyte)	41	22
Thymectomized (10) (high lymphocyte)	56	15
Sham-operated (15)	50	18

[a] Number of animals per group.
[b] Titer expressed as the reciprocal of the highest positive dilution.

were obtained from pools of serum collected 13 days after infection. Serum from the thymectomized group was positive at a greater dilution than the serum collected from the sham-operated animals at a time when the percentage of parasitemia was higher in the thymectomized group. In another group of rats fluorescent antibody titers were determined on individual serum samples collected 11 days after infection. The geometric mean of titers for each group is shown in Table III. No significant difference in fluorescent antibody titers was noted.

Discussion. These studies in neontally thy-

mectomized rats demonstrate an impaired host-response to *P. berghei* infection and are in agreement with earlier observations (14). Reinfection of the thymectomized animals failed to demonstrate the continued presence of the impaired host-resistance. The variable response noted in thymectomized rats could be largely eliminated by dividing the animals into high and low lymphocyte groups. Small thymic remnants were detected in a greater number of rats in the high than in the low lymphocyte group. This offers some explanation for the variability noted in unselected thymectomized animals.

The effect of neonatal thymectomy in rodents has been studied extensively (18). It is accepted that the primary effect is on cellular immunity as manifested by delayed skin homograft rejection and impaired delayed type hypersensitivity reactions. This effect in the rat has been documented (10, 11). Conflicting evidence is available concerning the effects of neonatal thymectomy on the antibody response in rats (10, 12, 13, 18). Immunoglobulin levels are not depressed. However, some evidence indicates that the production of a specific class of immunoglobulin is selectively inhibited (12), but this was not confirmed by other workers (13). In view of these findings the importance of thymic-dependent antibody production is uncertain.

The exact mechanism by which thymectomy alters the host-response to *P. berghei* infection is not known. A nonspecific decrease in the phagocytic capacity of the reticuloendothelial system could explain the higher parasitemias noted in the thymectomized animals, but this is unlikely in view of the increased rate of colloidal carbon clearance noted in thymectomized rats (Fig. 1). These results are consistent with the earlier report of enhanced clearance of colloidal carbon in thymectomized rats after an initial loading dose (19). Another possible explanation might be a decreased antibody response to the parasite. In these studies the measurable antibody level in thymectomized rats infected with *P. berhgei* was equal to or greater than the antibody level in sham-operated groups (Table III). Although it is recognized that the techniques used to measure the antibody response in these studies might be unsatisfactory for the detection of a specific type of immunoglobulin, it seems unlikely that the increased parasitemia (Fig. 2) and higher percent mortality (Table II) can be attributed to imparied antibody production. No direct measurement of cellular immunity is available but the effect of neonatal thymectomy on this system is widely accepted. The impairment of cellular immunity could conceivably result in increased susceptibility to the effects of *P. berghei* infection and in the above studies an alternative explanation is not available. These experiments support the concept that cellular immunity participates in the development of acquired resistance in *P. berghei* infected rats.

Summary. Neonatally thymectomized rats infected with *P. berghei* develop higher percentage parasitemias and have a higher percentage mortality than sham-operated animals. The degree of lymphocyte suppression in a thymectomized group can be used as an index of impairment of the host response to infection. The increased morbidity and mortality in thymectomized rats could not be explained on the basis of decreased phagocytic activity or antibody production. These studies suggest that thymic-dependent cellular immunity is important in the development of acquired resistance to *P. berghei* infection.

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Prevention of Experimental Allergic Encephalomyelitis by Combination of Epinephrine and a Phenothiazine Derivative, Propiomazine* (33969)

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Experimental allergic encephalomyelitis (EAE) is an experimental autoallergy which has similarities to certain human disease. One such disease is acute disseminated encephalomyelitis that sometimes follows viral infections such as mumps or vaccinia. The disease cannot be transferred by serum from a sensitized animal, but can be transferred by sensitized lymphoid cells (1). The possible role of humoral antibody in the disease cannot be ruled out (2). The disease has been prevented by treatment with cortisone (3), corticotropin (4), and 6-mercaptopurine (5).

In other studies epinephrine given to newborn rabbits inhibited development of lymphoid tissue and humoral antibody but did not inhibit homograft rejection (6). A phenothiazine derivative, propiomazine (Largon, Wyeth), known to possess antihistaminic and other protective properties (7), produced similar results; moreover, there was prolonged homograft survival in newborn rabbits (8). When given alone, neither epinephrine nor propiomazine produced these effects in adult rabbits; however, the two drugs given as a mixture to adult rabbits inhibited production of humoral antibody (9). In the latter studies peripheral leukocyte counts (total and differential) and serum complement levels were unaltered by the drug mixture. The above prompted further study of the effects of the drugs on a tissue reaction such as EAE which is considered to be mediated primarily by cell bound antibody (1).

Materials and Methods. Thirty-six adult rabbits weighing 1.5–2 kg were used. These were divided into 3 groups of 12 each. The first was given an initial daily intraperitoneal injection of 20 mg of propiomazine and 0.5 mg of epinephrine for a period of 3 weeks. At the end of this time, the dose was increased to 40 mg of propiomazine and 1.0 mg of epinephrine for an additional 2 weeks prior to sensitization and until completion of the experiment. The second group received intraperitoneally 40 mg of propiomazine and 1.0 mg of epinephrine on the day of injection of antigen and daily throughout the duration of

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