α-glucosidase, and phosphatase activities. The enzyme was inhibited by galactose (50 percent inhibition at 25 m*M* galactose and 2 m*M* substrate) and by *p*-hydroxymercuribenzoate (50 percent at $10^{-5}M$) but not by 1 m*M* *N*-ethylmaleimide. The inhibition by *p*-hydroxymercuribenzoate could be partly reversed by further addition of dithiothreitol.

The effect of temperature on enzymatic activity is shown in Figs. 1 to 3. In an experiment not shown in these figures, it was observed that the rate of hydrolysis of *p*-nitrophenyl-β-galactoside, when measured at 25° or 37°C in a buffer at *p*H 4, was almost linear for 90 minutes. Furthermore, only little deviation from linearity was obtained when enzyme and substrate were incubated at *p*H 4, for 10 minutes at temperatures up to 45°C (Fig. 1). It was, however, observed that at neutral *p*H, the salmon liver β-galactosidase was inactivated at temperatures above 20°C (Fig. 2, curve A). Thus, after 10 minutes at 30°C, only about two-thirds of the activity remained, and at 40°C, only one-third. This thermal inactivation at neutral *p*H was not prevented by the addition of substrates (2 m*M* *p*-nitrophenylgalactoside or 10 m*M* lactose), product (up to 50 m*M* galactose), or 1 m*M* dithiothreitol. In an experiment comparable to that shown in Fig. 2A, but in which the enzyme was first incubated without substrate for 10 minutes at *p*H 4, the respective losses at 30°C and 40°C were 10 and 25 percent of the β-galactosidase activity. For comparison, the effect of temperature on the activity of rat liver β-galactosidase (*3*) is shown in Fig. 2, curve B. Under similar experimental conditions all the activity of the latter enzyme was recovered at 40°C, but inhibition ensued at higher temperatures. [Similar results for the rat-liver enzyme have been reported (*4*).] It has also been shown that β-galactosidase from *Escherichia coli* lost no activity when heated for 30 minutes at 40°C, between *p*H 6 and 9 (*5*).

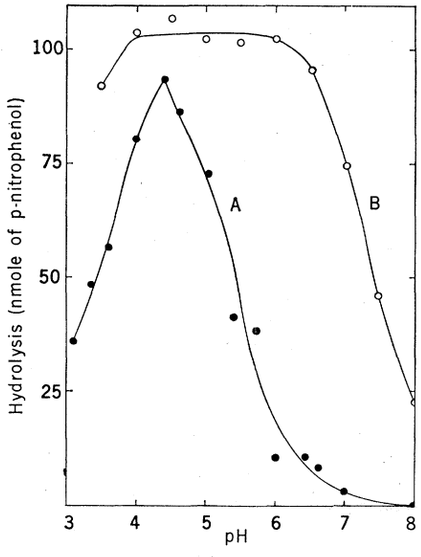


Fig. 3. Effect of *p*H on the thermal stability of β-galactosidase from salmon liver and rat liver. Mixtures containing 25 μmole of phosphate-citrate buffer at the specific *p*H values, enzyme, and water (in a volume of 0.35 ml) were incubated without substrates for 10 minutes at 48°C. Then 1 μmole of *p*-nitrophenyl-β-galactoside and 125 to 150 μmole of sodium citrate buffer, *p*H 4.0, were added. The tubes were further incubated for 10 minutes (at 28°C for the salmon liver enzyme and at 37°C for the rat liver enzyme). After addition of 0.3 ml of 1*N* NaOH and 2.2 ml of 0.25*M* glycine-carbonate buffer, *p*H 10, the color was read at 420 nm. Curve A represents 135 μg of salmon liver β-galactosidase; curve B represents 550 μg of rat liver β-galactosidase.

The difference in the response of salmon liver β-galactosidase to temperature at *p*H 4 and at *p*H 7 is amplified by the data shown in Fig. 3, curve A. The enzyme shows maximum thermal stability at about *p*H 4, but at *p*H values below or above this value the enzyme is rapidly inactivated at elevated temperature (Fig. 3, curve A). For comparison, the thermal responses of rat liver β-galactosidase, are shown in Fig. 3, curve B. Practically all the activity of the latter enzyme was recovered after 10 minutes at 48°C from *p*H 3.5 to 6.5.

The greater thermal lability of the salmon liver β-galactosidase at neutral *p*H requires special comment. Liver β-galactosidase is of lysosomal origin, with an optimum activity at about *p*H 4 (at *p*H 6 the rate of hydrolysis is 20 percent that of the optimum and at *p*H 7 about 10 percent). Considerable evidence has accumulated that the main function of the lysosomes involves the digestion of a variety of substrates of extra- or intracellular origin (*6*). Since many lysosomal enzymes have very low activities at neutral *p*H, it must be assumed that the lysosomes have an acidic microenvironment, separated from the rest of the cell. If so, the β-galactosidases of either salmon liver or rat liver exist in the lysosomes in an environment imparting to them their maximum thermal stability. If the lysosomes rupture or leak, this enzyme will spill into the cell sap, where it is a "foreign" protein which must be inactivated to prevent it from damaging the cell contents. The neutral or slightly alkaline *p*H of the cell sap will facilitate such an inactivation.

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**References and Notes**

1. S. Gatt and M. M. Rapport, *Biochim. Biophys. Acta* **113**, 567 (1966); F. B. Jungalwala and E. Robins, *J. Biol. Chem.* **243**, 4258 (1968); A. J. Furth and D. Robinson, *Biochem. J.* **97**, 59 (1965); D. Robinson, R. G. Price, N. Dance, *Biochem. J.* **102**, 525 (1967); F. Chytil, *Biochem. Biophys. Res. Commun.* **19**, 630 (1965).
2. For reviews on the effect of temperature on protein and enzyme stability, see S. Grisolia, *Physiol. Rev.* **44**, 657 (1964); A. S. Troshin, Ed., *The Cell and Environmental Temperature* (Pergamon Press, New York, 1967); C. L. Prosser, Ed., *Molecular Mechanisms of Temperature Adaptation* (AAAS, Washington, D.C., 1967).
3. The rat liver β-galactosidase was prepared by a method similar to that used with salmon liver except that the treatment at *p*H 4.5 was omitted. Protein precipitating between 40 and 60 percent with ammonium sulfate was collected, dialyzed against 10 m*M* sodium phosphate, *p*H 7.0, and stored at —20°C.
4. T. J. Langley and F. R. Jevons, *Arch. Biochem. Biophys.* **128**, 312 (1968).
5. K. Wallenfels, M. L. Zarnitz, G. Laule, H. Bender, M. Keser, *Biochem. Z.* **331**, 459 (1959).
6. C. de Duve, in *Subcellular Particles*, T. Hayashi, Ed. (Ronald Press, New York, 1959), p. 128; ——— and R. Wattiaux, *Ann. Rev. Physiol.* **28**, 435 (1966).
7. This investigation was performed on the R.V. *Alpha Helix*, as part of its Bering Sea Expedition (supported by NSF grant GB 7173). Phase IV of this Expedition consisted of an investigation of the biochemistry and physiology of salmon during the spawning season. It was supported in part by NIH grants NB-02967 and GM-12310. I thank Dr. A. A. Benson for review of the manuscript and for discussions.
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## Protective Effect of Antilymphocyte Serum on Mice Infected with Plasmodium berghei

Abstract. *Rabbit antiserum to mouse lymphocytes prolonged the survival of mice infected with* Plasmodium berghei. *Since antilymphocyte serum has a well-defined, potent immunosuppressive effect, the immune response of the host to the parasite actually may contribute to the death of the animal with an acute malarial infection.*

*Plasmodium berghei* infection in mice is fulminating (*1*), death of the animals being due to the development of high parasitemia accompanied by profound anemia (*2*). While most observers attribute the anemia to infection and subsequent destruction of

Table 1. Parasitemia on the 5th day of *P. berghei* infection in treated and control groups of mice; RAMLS, rabbit antiserum to mouse lymphocytes; NRS, normal rabbit serum.

| Treatment | Group size (No.) | Parasitemia (%) | | |
|---|---|---|---|---|
| | | Mean | Range | $P$* |
| Thymectomy + RAMLS | 10 | 7.9 | (0.2 – 50.6) | $< .001$ |
| Sham + NRS | 9 | 45.8 | (32.5 – 71.9) | |
| RAMLS | 10 | 17.7 | (0.4 – 69.7) | $< .02$ |
| NRS | 7 | 48.1 | (39.6 – 61.8) | |

* Wilcoxon test.

circulating erythrocytes (*3*), others have suggested that the immune reaction of the host to the parasite contributes to the pathologic process (*4, 5*). To ascertain what effect suppression of the immune response would have on an experimental malarial infection, we subjected mice to treatment with rabbit antiserum to mouse lymphocytes and adult thymectomy before infecting them with *P. berghei*. Antilymphocyte serum has been used as a potent immunosuppressive agent of both humoral and cellular immune mechanisms in mice; thymectomy of the adult mouse has been shown to potentiate the immunosuppressive effects of this antiserum (*6*).

Male white mice (strain CFW, NIH animal colony; aged 6 to 8 weeks; 18 to 22 g) were thymectomized and allowed to recover for 2 weeks. Littermates were given a sham operation at the same time, and they had an identical recovery period. The rabbit antiserum to mouse lymphocytes was prepared as described (*6*) and had a lymphocyte agglutinin titer of 1:512. This serum prolonged the survival of $C_3H/He$ skin grafts to A/Jax mice, when given intraperitoneally in a dose of 0.25 ml daily for 5 days, the median survival time being 10.2 ± 0.3 days for the untreated and 17.4 ± 0.4 days for the treated mice. Treatment was begun in 20 thymectomized animals with antilymphocyte serum (0.25 ml/day) and in 21 sham-operated animals with normal rabbit serum (0.25 ml/day) for 5 days. Two days later, all animals plus a group of 30 nonoperated, untreated controls were infected with *P. berghei* (NK-65 strain). The inoculum consisted of $10^7$ parasitized erythrocytes administered intraperitoneally.

Numbers of dead animals were counted twice daily, and randomly selected mice were examined at autopsy for the presence or absence of residual thymic tissue. None was found in any thymectomized animal. No attempt was made to evaluate actual suppression of antimalarial antibody, for immunofluorescent antibody against *P. berghei* in these mice is not detectable during the acute infection. In that bacterial endotoxins will produce a nonspecific resistance to *P. berghei* infections in mice (*7*), we examined our preparations of antilymphocyte serum for the presence of pyrogen. No fever was produced by 1.0-ml amounts of the antiserum when it was injected into normal rabbits.

Significant delay in mortality ($P <$ .01, Smirnov test) was observed in the group of thymectomized mice treated with antilymphocyte serum (Fig. 1). The animals that received the sham operation and the normal rabbit serum as well as the untreated controls died at the same rate.

To define more precisely what role thymectomy, the antiserum, or the combination was playing in the protective effect, the following experiment was performed. Animals were thymectomized or sham-operated as in the previous experiment: of 60 thymectomized mice, 20 were treated with antilymphocyte serum and 40 were left untreated; of 56 sham-operated animals, 20 were treated with normal rabbit serum and 36 were left untreated; of 61 nonoperated controls, 20 were treated with the antiserum, 20 with normal serum, and 21 were left untreated. All groups were then infected with *P. berghei*, and the results are shown in Fig. 2. Again, significant protection ($P < .01$) was seen in the thymectomized group treated with antilymphocyte serum, as compared with any of the other control groups. The nonoperated group treated with antilymphocyte serum alone received significant protection ($P < .01$) when compared to nonoperated animals treated with normal rabbit serum. Thymectomy alone had no protective effect and did not significantly potentiate the effect of antilymphocyte serum.

During the latter experiment, daily blood smears for evaluation of parasitemia were obtained from ten randomly selected animals in each group. The smears were stained with Giemsa, and the percentage of parasitized erythrocytes was ascertained after examination of 500 cells on each slide. No attempt was made to differentiate multiply infected cells or to judge different stages of development of the parasite. After the mortality data had been eval-

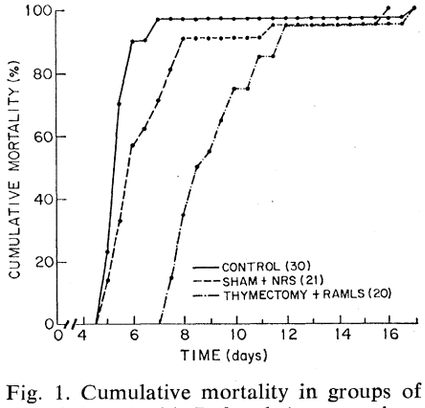


Fig. 1. Cumulative mortality in groups of mice infected with *P. berghei* at zero time. Control animals received no treatment. Thymectomized animals were treated with rabbit antiserum to mouse lymphocytes (RAMLS); and sham-operated animals were treated with normal rabbit serum (NRS). Numbers of animals in each group are in parentheses.

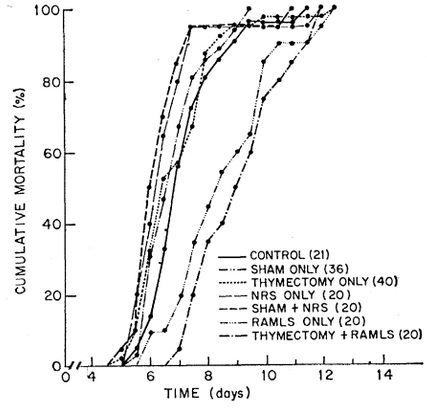


Fig. 2. Cumulative mortality in treated and control groups of mice infected at zero time. Unoperated animals received either rabbit antiserum to mouse lymphocytes (RAMLS), normal rabbit serum (NRS), or no treatment. Thymectomized animals received either RAMLS or no treatment. Sham-operated animals received either NRS or no treatment. Numbers of animals in each group are in parentheses.

uated, it was decided to compare parasitemias on day 5 in animals from four of the groups: thymectomized animals treated with antilymphocyte serum were compared with those in the sham-operated group treated with normal serum; also, the group treated with antilymphocyte serum alone was compared with that treated only with normal rabbit serum (Table 1). There was a significant ($P < .02$, Wilcoxon test) depression of parasitemia in each of the groups treated with antilymphocyte serum as compared with its appropriate control.

These studies demonstrate the protective effect of the administration of antilymphocyte serum on *P. berghei* infection in mice. It appears that adult thymectomy, in this system, has little additive effect. This fact suggests that the suppression of humoral immune mechanisms may be more important than the inhibition of cellular immunity in the observed protection, for adult thymectomy seems mainly to potentiate the effect of antilymphocyte serum on cellular immune mechanisms (*6*). With regard to the role of humoral immune factors in malaria, McGhee (*4*) presented evidence that the development of the anemia in ducklings with malaria could not be correlated with parasitemia, and he speculated that at least part of the observed destruction of erythrocytes might be due to the concomitant development of an autoimmune reaction to host erythrocytes. Similarly, the thrombocytopenia regularly seen in malaria (*8*) seems also related in some way to the development of the immune response (*9*).

Thus, the results of our experiments might be interpreted as follows. Impairment of the immune response is actually of transient benefit to the host with a fulminating plasmodial infection in that it permits longer circulation of erythrocytes and ultimate prolongation of survival. If such were the case, one might expect that the degree of parasitemia in the immunosuppressed animals should be equal to that in the control group; but we have shown that parasitemia is suppressed (Table 1). Hence, antilymphocyte serum may have an intrinsic antiparasitic effect.

Using a different model system, Wright (*10*) has described the effect of neonatal thymectomy in hamsters infected with *P. berghei*. In this species, the infection is usually fatal in 6 to 12 days. Animals thymectomized at birth survived significantly longer (death occurring between 19 and 25 days), even though their hemoglobin concentrations and parasite levels were similar to those of nonthymectomized animals earlier in the course of the infection. Wright proposed that neonatal thymectomy inhibited or delayed production of immune responses which lead, in control animals, to microembolization of the cerebral capillaries with agglutinated parasitized erythrocytes.

Brown *et al.* (*11*) described the effect of neonatal thymectomy on the course of *P. berghei* infection in rats. In the rat, immunity to *P. berghei* develops rapidly, and clearing of parasitemia occurs with ultimate survival of most animals. Among adult rats that were thymectomized at birth the mortality was higher and the duration of parasitemia was longer. Since cell-mediated immunity is most strikingly depressed by neonatal thymectomy, Brown *et al.* postulated that such immune mechanisms may play a part in the resolution of malarial infections.

Thus, these studies show that the immune response itself contributes significantly to the death of animals undergoing a fulminating malarial infection. But, in those infections where the immune response eventually brings about control of the parasitemia, immunosuppression is deleterious.

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**References and Notes**

1. J. P. Thurston, *Exp. Parasitol.* **2**, 311 (1953).
2. B. T. Wellde, N. T. Briggs, E. H. Sadun, *Mil. Med.* **131**, 859 (1966).
3. H. W. Cox, *ibid.*, p. 1195; J. N. George, E. F. Stokes, D. J. Wicker, M. E. Conrad, *ibid.*, p. 1217.
4. A. B. McGhee, *J. Infec. Dis.* **107**, 410 (1960); *Amer. J. Trop. Med. Hyg.* **13**, 219 (1964).
5. A. Zuckerman, *Nature* **185**, 189 (1960); *Mil. Med.* **131**, 1201 (1966).
6. A. P. Monaco, M. L. Wood, P. S. Russell, *Science* **149**, 432 (1965).
7. L. K. Martin, A. Einheber, E. H. Sadun, R. E. Wren, *Exp. Parasitol.* **20**, 186 (1967); R. R. MacGregor, J. N. Sheagren, S. M. Wolff, *J. Immunol.* **102**, 131 (1969).
8. G. J. Hill, V. Knight, G. M. Jeffery, *Lancet* **1964-I**, 240 (1964).
9. J. N. Sheagren, N. R. Shulman, W. A. Sodeman, Jr., *Clin. Res.* **16**, 313 (1968).
10. D. H. Wright, *Brit. J. Exp. Pathol.* **49**, 33 (1968).
11. I. N. Brown, A. C. Allison, R. B. Taylor, *Nature* **219**, 242 (1968).
12. The technical assistance of I. Barrett, J. Edelin, and D. Lawless is acknowledged. Dr. D. Alling assisted with the statistical analyses.

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## Queuing Behavior of Spiny Lobsters

Abstract. *Autumnal mass migrations of spiny lobsters,* Panulirus argus, *involve diurnal movements of thousands of individuals in single-file queues. Initiation, posture, and alignment of a queue can be effected entirely by tactile cues received through antennular inner rami, pereiopods, and antennae. Since spiny lobsters queue when deprived of shelter, this behavior may serve a defensive function. Specimens captured while migrating maintain the queue indoors for up to several weeks, whereas at other times the queue lasts only a few hours. Hence, the migratory behavior probably depends in part upon environmentally induced neurohormonal changes.*

The occurrence of "columns" or "trains" of spiny lobsters, *Panulirus argus*, moving over open areas was reported as early as 1922 (*1–3*), but the extent and significance of the phenomenon has remained inadequately described. In autumn, thousands of lobsters migrate diurnally in parallel single-file queues across shallow areas near Bimini, Bahamas, and the Florida east coast (*4, 5*). This activity is remarkable since spiny lobsters are usually seclusive by day, remaining in crevices on the reef and emerging at night to feed.

The widespread occurrence, periodicity, and large numbers of individuals involved, as well as the stereotyped behavioral character of the queues, clearly defines this phenomenon and suggests previously unrecognized significance of these migrations in the life history of the species. Furthermore, the mode of mass movement, which outwardly resembles migrations of army ants and certain bird flocks, and its abrupt nature make it unique among benthic marine crustaceans (*3*). I now present some major behavioral characteristics of the single-file formation,