Interferon Induced by Plasmodium berghei

Abstract. *An inhibitor of virus is demonstrable in the serums of mice infected with* Plasmodium berghei. *The titer of the inhibitor, detectable within 10 hours after injection of parasitized blood, increases rapidly until 30 to 40 hours when it levels off or decreases slightly before reaching a plateau. The factor that induces production of the antiviral substance is not present in the plasma of the infected blood, and the inhibitor is not detectable in serums of mice injected with nonparasitized mouse erythrocytes. The inhibitor fulfills the essential requirements of an interferon.*

Although interferon was first demonstrated in association with viral infection (*1*), a large number of other types of interferon inducers have since been recognized (*2*). Among these are other intracellular microbes, such as *Chlamydia* (psittacosis-trachoma group) (*3*), *Rickettsia* (*4*), and a protozoon, *Toxoplasma gondii* (*5*). Because the intracellular site seems to be a significant factor in interferon induction by infectious agents, another intracellular protozoon, *Plasmodium berghei,* which causes malaria infection in mice, was examined for its capacity to induce interferon.

The NYU-2 strain of *P. berghei* (*6*) was maintained in Swiss mice (20 to 25 g, NMRI strain) by intraperitoneal passage of 2×10^7 parasitized red blood cells harvested from infected mice 5 to 6 days after injection. Inocula were prepared according to the methods of Hillyer and Diggs (*7*). At intervals, groups of seven mice were bled from the axilla, the blood was pooled, and serum was separated. The serums were then centrifuged at 500*g* for 15 minutes to remove any red cells and parasites. Parasitemia was determined by a standard method of counting parasitized red cells. Mice injected with normal erythrocytes furnished the control serums. Serums were assayed for viral inhibition in strain L-929 cells grown in Eagle's minimum essential medium (MEM) supplemented with fetal bovine serum (10 percent) plus penicillin, streptomycin, achromycin, and mycostatin. A plaque reduction method (*8*) was used, with 50 plaque-forming units of vesicular stomatitis virus. The titer of antiviral activity was expressed as the reciprocal of the dilution of the preparation that gave 50 percent reduction in the number of plaques.

Observations made during a representative experiment are shown in Fig. 1. Death occurred as usual between day 6 and day 8. Antiviral activity was demonstrable 10 hours after infection, and by 20 hours the titer was 72 unit/ml. The peak of 250 unit/ml was reached by 35 hours; the titer then decreased but remained between 60 to 80 unit/ml for the rest of the survival period. In a confirmatory experiment, 158 units of antiviral activity per milliliter were present in the serum 33 hours after injection, and 160 unit/ml 112 hours after injection. Antiviral activity in control specimens was low during the first 10 hours, but essentially absent later.

To characterize this antiviral substance, tests were carried out with normal mouse serum and mouse serum interferon induced by Newcastle disease virus as controls. The antiviral substance was not sedimented after centrifugation at 105,000*g* for 90 minutes. Its activity was cell-associated; it was not removed by washing the cells before challenging with virus. It did not act directly against the virus; incubation of vesicular stomatitis virus with a serum specimen at room temperature for 30 minutes did not reduce the titer of virus. Trypsin abolished at least 90 percent of the activity, and heating at 60°C for 1 hour completely inactivated it. The activity remained stable overnight at *p*H 2. Besides being active against vesicular stomatitis virus in L cells, the mouse serum also effectively protected the same cells against Semliki Forest virus. That antiviral activity was not demonstrable in an established line of monkey kidney cells (MK2) challenged with vesicular stomatitis virus indicated species specificity. Thus, it appears that the antiviral substance in the serums of mice infected with a malarial parasite meets the criteria to be called interferon.

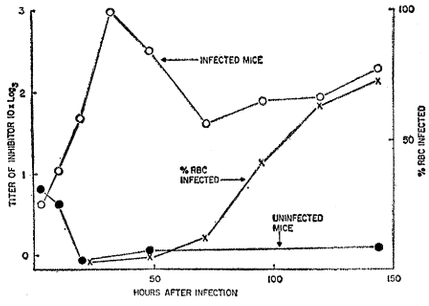

Fig. 1. Parasites and antiviral inhibitor in serums of mice infected with *P. berghei.*

Table 1. Titer of interferon in serums of mice injected with citrated whole blood, plasma, or centrifuged sediment of whole blood, from malaria-infected mice.

Hours after injection	Units of interferon per milliliter of serum induced by		
	Whole blood	Plasma	Sediment
7	24	< 3	16
34	243	< 3	198
144	108	< 3	82

Since serums of mice injected with nonparasitized red cells did not show significant antiviral activity, the possibility can be ruled out that the interferon was induced by red cells or other components of uninfected blood. To exclude the possibility that the factor inducing production of interferon was present in the plasma of infected blood, parasitized blood was centrifuged at 17,500*g* for 30 minutes, to separate red cells from plasma. The latter was saved, and the sediment was further subjected to two cycles of washing with phosphate-buffered saline (PBS) followed by a centrifugation at the same speed. After the final centrifugation, the sediment was suspended in the original volume of PBS. Both the plasma and suspended sediment were diluted to the same degree so that in the sediment there were 2×10^7 parasitized red cells per 0.2 ml; and each was injected into groups of mice, which were then examined for serum interferon as described. Only the sediment induced interferon to a degree similar to that induced by the infected whole blood (Table 1). Thus, the interferon is not induced by a factor present in the plasma of infected blood, such as a virus, or products of malarial infection, but by a factor that sediments with the red cells, presumably the plasmodium.

In considering the question of where the plasmodium-induced interferon is produced, it must be recalled that tissue stages of the parasite are said not to result from blood-induced infections in any of the mammalian species of *Plasmodium.* Infection of fixed tissue cells, therefore, cannot account for the production of the interferon. In considering whether interferon is produced by parasitized circulating red cells, present concepts relate interferon production to the cellular genome. Although no direct evidence is available concerning the ability of nonnucleated red cells to pro-

duce interferon, this possibility seems unlikely. Evidence supporting such speculation was obtained by Guggenheim *et al.* (*9*) who showed that chick (nucleated) red cells, unlike other chick cells, were not stimulated by Sendai virus to produce interferon. Preformed interferon released by injection of endotoxin into mice is characterized by a peak at 2 hours and a rapid fall in titer. The finding of a late peak of plasmodium-induced interferon and its persistence in the serum appears to preclude release of existing interferon as a result of injection. The potential role of interferon in plasmodial infection has been emphasized also by findings (*10*) that various interferon inducers exert a protective effect in mice against infection with *P. berghei*. In view of the observation that interferon inhibits other nonviral intracellular microorganisms such as *Chlamydia* (*11*) and *Toxoplasma* (*12*), interferon apparently possesses a wider and more complex spectrum of activity than is now understood. Investigations of the relation between interferon and *Plasmodium* may not only reveal the role of interferon in malarial infection, but may further lead to the understanding of the action of interferon.

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Epinephrine: Effect on Uptake of Iodine by Dispersed Cells of Calf Thyroid Gland

Abstract. *In isolated thyroid cells* l-*epinephrine (0.1 and 10.0 micrograms per milliliter), norepinephrine and isoproterenol consistently stimulated the accumulation and organic binding of iodine. The effect was partially inhibited by phentolamine, but not propranolol, and hence may be mediated by alpha receptors. Theophylline did not mimic or enhance the epinephrine effect, suggesting that the latter may not result from activation of adenyl cyclase.*

3′,5′-Cyclic adenosine monophosphate may be a mediator of the action of several hormones, notably vasopressin, adrenocorticotrophic hormone, adrenomedullary hormones (*1*), and parathyroid hormone (*2*). In thyroid homogenates (*3*) and isolated thyroid cells (*4*), thyrotropin (TSH) has been shown to stimulate the activity of adenyl cyclase, an enzyme that catalyzes cyclic adenosine monophosphate (AMP) synthesis. The dibutyryl ester of cyclic AMP, like TSH, increases the incorporation of labeled orthophosphate into the phospholipids of thyroid slices (*5*) and theophylline, an inhibitor of cyclic AMP hydrolysis, enhances the stimulatory effect of TSH on thyroid iodine metabolism (*6*). Several reports indicate that dispersed thyroid cells retain both the essential features of hormonal synthesis and the response to TSH which are characteristic of the intact gland (*7, 8*). Therefore, we have investigated the effect of *l*-epinephrine, a known stimulator of adenyl cyclase activity in other tissues (*1, 9*), and of related sympathetic amines, on the uptake and metabolism of iodine by dispersed cells of calf thyroid gland.

Fresh calf thyroids were collected and stored on ice during transport to the laboratory. Within 1 hour the glands were trimmed of fat and fascia, minced, and then treated with trypsin for 4 hours (*7*). The material collected between the 2nd and 4th hour of trypsin treatment was centrifuged and washed twice with 50 volumes of Earle's solution. This material was then suspended in Earle's solution supplemented with 2 μc of ^{131}I and 3 μg of ^{127}I as sodium iodide per milliliter. The suspension was allowed to stand for several minutes, during which time the aggregated cells and debris sedimented. The suspended cells, now dispersed in 50 to 100 volumes of Earle's solution (18 to 20 × 10^6 cell/ml), were separated by decantation. Portions (2 ml) were added to incubation vessels in which various substrates had been added in a volume of 0.5 ml. Incubations were carried out in duplicate for 45 minutes in a Dubnoff shaker at 37°C with either a mixture of 95 percent O_2 and 5 percent CO_2 or 100 percent O_2 as gas phase. In each experiment two or three vessels containing cells which had been boiled for 5 to 7 minutes were incubated under the same conditions. After incubation, measured portions were centrifuged, the supernatant was discarded, and the ^{131}I content of the sedimented cells was measured in a well-type scintillation counter. Accumulated ^{131}I was related

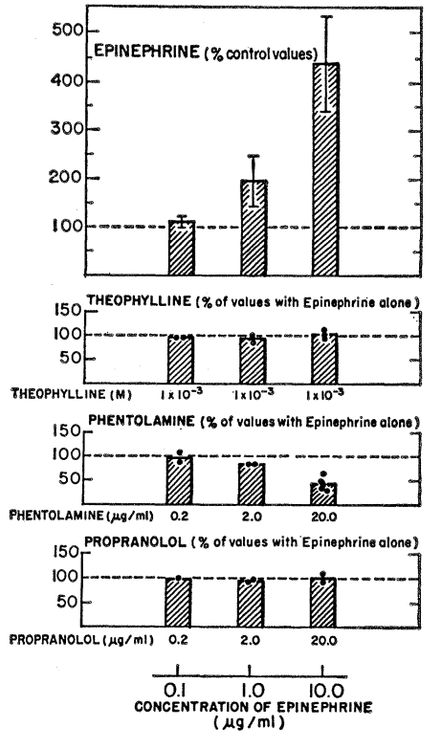

Fig. 1. The effect of epinephrine, theophylline, and adrenergic blocking agents on the uptake of ^{127}I by isolated thyroid cells. In this and Figs. 2 and 3, results are from incubations carried out in Earle's solution for 45 minutes at 37°C, with 100 percent O_2 as gas phase. Values shown in the upper panel represent the mean ± S.D. of results obtained in 12 experiments. The stimulation of iodine accumulation induced by epinephrine was consistently associated with an increase in the proportion of accumulated iodine incorporated into organic forms and an increase in organic iodine present as thyroxine. In the three lower panels, each point represents the results of a single experiment in which duplicate or triplicate vessels were incubated.