association of twinning with both decreased right-handedness and reduced college entrance (Table 1). In the elementary school sample, twin-birth subjects had a mean LQ that was 16 points lower than that of single-birth subjects ($z = 1.79$, $P < .05$, one-tailed test). A higher incidence of left-handedness among both monozygotic and dizygotic twins has also been found in Western studies (*7, 8*). Furthermore, one study located eight pairs of twins with discordant handedness of which one member was institutionalized for mental retardation and the other was not. In all cases, it was the left-handed member who was institutionalized (*8*). Although we found comparable handedness between twin-birth and single-birth subjects in our college sample, it is important to note that the percentage of twin-birth subjects in the college sample was less than half of that in the elementary school sample (chi square = 10.31, $P < .01$). Since subjects in our college sample were highly selected for academic successes, the pattern of results supports the earlier Western findings in indicating that twinning may sometimes affect both handedness and intelligence. The relatively high risk status of twinning during both prenatal and perinatal periods is well known (*9*). Among a variety of possible factors, such as intrauterine crowding, long labor, and low birth weight, which may be particularly responsible for possible cerebral impairment, remain to be determined.

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Malaria: Successful Immunization Against the Sexual Stages of *Plasmodium gallinaceum*

Abstract. *Gametocyte infectivity and oocyst development of the avian malaria parasite,* Plasmodium gallinaceum, *can be reduced or eliminated in mosquitoes by immunizing the chickens on which the mosquitoes feed with infected red blood cells that have been treated with formalin or x-rays. Protection of the mosquito appears to be related to the immobilization of the microgametes in its gut and is associated with the immunoglobulin G fraction of serum.*

Successful immunization with malarial sporozoites and merozoites (*1*) is thought to be dependent upon the vulnerability to serum factors of the invasive asexual stages of these protozoan parasites when they are outside vertebrate host cells. Malarial parasites are also exposed to the extracellular environment in another phase of development when the sexual parasites, the gametocytes, shed their erythrocyte membranes after being ingested by a mosquito. In the gut of a mosquito vector, gametocytes give rise to spermlike male gametes and nonmotile female gametes which fuse; the resulting ookinetes (zygotes) penetrate the gut epithelium to produce oocysts. It is the oocyst which ultimately produces sporozoites capable of infecting a vertebrate host to complete the cycle of transmission when the mosquito feeds again.

That host factors can interfere with the capacity of gametocytes to produce oocysts in mosquitoes has been noted (*2*), but not pursued. In this report I describe an immunity induced in chickens which affects only the sexual stages of the avian malaria parasite, *Plasmodium gallinaceum*, within the gut of the mosquito vector.

New Hampshire Red chickens were immunized with red cells infected with *P. gallinaceum* that had been inactivated by treatment with formalin (1 percent for 30 minutes; the cells were then washed twice and resuspended in saline) or with

Table 1. The effect of various immunization schedules on oocyst development on the gut of mosquitoes. Parasitized erythrocytes (2×10^9) were inactivated with formalin or x-rays and injected into chickens intravenously. The data for parasitemia are expressed as the numbers of oocysts per mosquito gut (mean of ten mosquitoes per chicken per day) when the mosquitoes fed upon chickens with parasitemias within the ranges indicated below and for decreasing (1 day after peak) parasitemia.

Number of weekly injections	No. of chickens	Parasitemia (% infected erythrocytes)*						Cumulative mean oocysts per gut for 5 days†
		≤ 0.4	0.5 to 5	6 to 40	41 to 85	Decreasing		
Formalin-treated antigen; avian host challenged with 10^5 parasites								
5	1	0	0	0	0	0		0
4	5	0	0	0.04	0.1	0.01		0.03
3	3	0	0.07	5.6	6.8	0.02		2.4
2	9	4.2	27	33	17	1.2		17
1	5	11	32	103	76	30		50
X-irradiated antigen; avian host challenged with 10^5 parasites								
4	2	0	0	0.2	0.7	0.07		0.19
3	7	0	0.4	1.9	2.7	0.3		1.1
2	5	0.08	2.9	3.9	5.5	4.5		3.4
1	1	9	58	94	41	11		43
Nonimmune control; avian host challenged with 10^5 parasites								
None	15	14	82	164	35	18		63
X-irradiated antigen; avian host challenged with bites of ten mosquitoes								
3	3	0	3.1	2.2	‡	1.7	0.2§	1.4
2	4	1.6	18	10	‡	4.4	0.2§	6.8
Nonimmune control; avian host challenged with bites of ten mosquitoes								
None	10	41	110	72	‡	77	39§	68

*Parasite count increased at a predictable rate with 3 days of increasing parasitemia prior to the peak. †Ten mosquitoes per chicken for each of 5 days of maximum oocyst production. ‡Maximum parasitemia in sporozoite-induced infections less than 40 percent. §Two days of decreasing parasitemia were recorded here.

x-rays (35 krad). Chickens weighing 200 to 350 g were injected intravenously with approximately 2×10^9 treated parasites (approximately 2×10^7 gametocytes) at 7-day intervals (see Table 1). Each chicken was then challenged 7 to 10 days after the final inoculation with either an injection of 10^5 untreated parasites or with the bites of ten infected mosquitoes. Parasitemia was monitored by taking daily blood samples that were examined as Giemsa-stained thin smears. As an indicator of gametocyte infectivity, *Aedes aegypti* mosquitoes were fed on chickens from the day parasites first appeared in the peripheral circulation to 2 days after peak parasitemia. Mosquitoes were dissected 7 days after feeding and oocysts growing on the mosquitoes' gut were stained with 0.5 percent Mercurochrome and counted.

The effect of the immunization of the chickens on the infectivity of the gametocytes in mosquitoes is shown in Table 1. In mosquitoes that ingested gametocytes from chickens receiving two or more inoculations few oocysts developed, whereas mosquitoes that ingested gametocytes from once-inoculated or nonimmunized control chickens developed extensive gut infections, primarily on the day preceding the day of highest asexual parasitemia. In control experiments, when chickens were inoculated with formalin-killed or x-irradiated uninfected blood cells weekly for 3 weeks prior to being challenged, the infectivity of the parasites in the mosquitoes was not affected.

Immunization had little effect on the course of the asexual infections in chickens; the appearance of circulating parasites was sometimes delayed 1 to 2 days, but maximum asexual parasitemias were comparable to control infections. Immunization did not affect gametocyte production; gametocyte counts relative to asexual parasites were similar in both immune and control chickens.

Immunization did not affect the functional integrity of circulating gametocytes; as long as the gametocytes remained enclosed within erythrocyte membranes they were potentially infective (Table 2). Erythrocyte-cloaked gametocytes from immunized chickens washed (*3*, *4*), resuspended in normal plasma, and fed to mosquitoes through a membrane (*5*) were infectious. In mosquitoes that ingested these washed gametocytes, the rate of infection (number of oocysts per mosquito gut) was similar to that in mosquitoes that ingested blood from nonimmunized chickens. When gametocytes that had been washed were resuspended in autologous immune plasma and then fed to mosquitoes, they produced few oocysts.

Table 2. Oocyst development in mosquitoes fed on gametocytes from immunized chickens after the infected erythrocytes had been washed and resuspended in normal or autologous immune chicken plasma.

Number of weekly injections	Parasitemia* (%)	Oocysts per gut (mean No.)†			
		Direct feeding‡	Resuspended in plasma§		Non-immune (expected)‖
			Autologous	Normal	
		Formalin-treated antigen			
4	15	0	0.05	163	164
4	22	0	0.5	106	164
4	60	0.1	0.6	45	35
5	31¶	0	0	3.8	18
		X-irradiated antigen			
2	56	0.9	7	84	35
3	18	0	0	76	164

*Percentage of infected red cells at the time of mosquito feeding. †Ten mosquitoes dissected per treatment. ‡Mosquitoes fed directly on the donor chicken. §Mosquitoes fed through a membrane. ‖The expected mean oocyst count from mosquitoes feeding directly on nonimmunized chickens (Table 1). ¶One day after the peak of parasitemia.

The precise nature of the reaction which prevents oocyst development in the gut of the mosquito is unknown, but one possible mechanism may be related to the behavior of male gametes from immunized chickens. Gametocytes undergo a number of characteristic changes when ingested by a mosquito; the same sequence of events occurs when a drop of infected blood is placed on a microscope slide (*4*). Within minutes, normally oval gametocytes round up and shed the erythrocyte plasmalemma. Exflagellation commences and the resulting male gametes remain active for more than 1 hour. Gamete behavior in blood drawn from immunized chickens is significantly modified. Gametocyte rounding and red cell membrane disintegration proceed normally. Exflagellation is initiated, but the male gametes are arrested prior to or just after release from the body of the gametocyte. Gametocytes from immunized or nonimmune chickens, when washed and resuspended in normal plasma or serum, exflagellate and the gametes sustain activity; gametocytes suspended in immune plasma or serum begin exflagellation, but male gametes are immobilized within seconds. This almost immediate immobilization of escaping or just-freed male gametes should severely limit fertilization and could explain the consequent reduction of oocysts in mosquitoes.

Results from several experiments (*6*) indicate that gamete immobilizing and transmission blocking activities are associated with host antibodies: (i) An ammonium sulfate precipitate of immune serum resuspended in normal serum gives a strong gamete immobilizing reaction and completely inhibits oocyst development in mosquitoes. Conversely, immune serum from which immunoglobulin G (IgG) had been precipitated with rabbit antiserum to chicken IgG has no immobilizing activity. (ii) Separation of a sodium sulfate precipitate of immune serum on a Sephadex G-200 column showed that gamete immobilizing activity is restricted to the IgG fraction. (iii) The gamete immobilization reaction is not complement dependent. Immune serum inactivated at 56°C for 30 minutes is equivalent to untreated immune serum in its ability to immobilize exflagellating or free male gametes and prevent the infection of mosquitoes fed through a membrane.

A vaccine capable of interrupting the cycle of malaria transmission by reducing or eliminating mosquito infections offers a new approach to the control of one of the major diseases affecting man. The feasibility of immunization against the sexual stages of the human malarias remains to be determined.

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