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10. Total unfractionated ν_1 consists of DNA fragments of 125 to 180 bp associated with the inner histones and variable amounts of H5 and H1. Dialysis of total ν_1 against 0.1*M* KCl results in a soluble fraction containing equimolar amounts of H4, H3, H2B, and H2A associated with DNA (~ 140 bp), and a precipitated fraction containing all of the histones including H5 and H1 associated with DNA fragments. The products of subfractionation are described in (*2*). Unless stated otherwise, the Raman spectra of ν_1 reported here refer to the KCl-soluble ν_1 fraction (standard sedimentation coefficient at 20°C in water, 11.4; molecular weight, 210,000 to 216,000).
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24. For an extensive review of the circular dichroic data for histones and chromatin, see G. D. Fasman, P. Y. Chou, and A. J. Adler [in *The Molecular Biology of the Mammalian Genetic Apparatus*, part A, P. O. P. T'so, Ed. (Elsevier/North-Holland, Amsterdam, in press)]. These authors also apply a predictive method to the amino acid sequences of the histones and calculate a maximal α-helix content for the inner histones of 36 percent (averaging their maximal values for the separate histones H4, H3, H2B, and H2A), with 22 percent β-sheet and 43 percent β-turn or random structure. Our results reported here do not support their prediction of considerable β-sheet structure. Whereas Fasman *et al.* incline toward the view that β-sheet–β-sheet interactions between the apolar regions of the inner histones might parallel those in such proteins as insulin and concanavalin A, we suggest that α-helix–α-helix associations between subunits, as in hemoglobin, would better explain our observed high α-helix and negligible β-sheet content in chromatin nu bodies. This view would be consistent with recent estimates of the secondary structure of H3-H4 tetramer (29 percent α helix and no β structure) and the H2A-H2B complex (37 percent α helix and no β structure) [T. Moss, P. D. Cary, C. Crane-Robinson, E. M. Bradbury, *Biochemistry* **15**, 2261 (1976); T. Moss, P. D. Cary, B. D. Abercrombie, C. Crane-Robinson, E. M. Bradbury, *Eur. J. Biochem.* **71**, 337 (1976)]. A comparison of these data with our own suggests the possibility that formation of the heterotypic tetramer or the inner histone octamer within ν_1, from the homotypic histone complexes, results in a net increase in α-helix content but no apparent increase in β structure.
25. We thank R. E. Harrington for analyzing the circular dichroic spectra of the inner histones. R. E. Harrington and A. L. Olins critically evaluated the manuscript. E. B. Wright helped in the preparation of materials. This work was supported in part by NIH grants AI 11855 (G.J.T.) and GM 19334 (D.E.O.) and by the Energy Research and Development Administration under contract with Union Carbide Corporation. The results of this investigation were presented at the 21st annual meeting of the Biophysical Society and were published in an abstract [B. Prescott, G. J. Thomas, Jr., D. E. Olins, *Biophys. J.* **17**, 114a (1977)].

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An Effective Immunization of Experimental Monkeys Against a Human Malaria Parasite, *Plasmodium falciparum*

Abstract. *This is the first report of successful immunization of experimental monkeys against a human malaria parasite,* Plasmodium falciparum. *Of the five owl monkeys* (Aotus trivirgatus) *used in this pilot study, two served as controls and the other three were immunized with* P. falciparum *antigen consisting primarily of mature segmenters containing fully developed merozoites. Two injections of antigen emulsified with Freund's complete adjuvant were administered intramuscularly 3 weeks apart. Three weeks after the second vaccination, all monkeys were challenged with the homologous strain of* P. falciparum. *The control monkeys died with high levels of parasitemia within 2 weeks of challenge. The three immunized monkeys survived and showed strong protection against* P. falciparum. *These results are encouraging for the possible future development of an effective vaccine against human malaria.*

Attempts to develop a vaccine against malaria began half a century ago but gave way to searches for new drugs during World War II and to antimosquito programs afterward. However, resistance to drugs and insecticides and lack of money have reduced the prospects of malaria eradication, and thoughts have again turned to the possibility of developing a malaria vaccine (*1*).

Four different kinds of vaccines are currently under investigation: exoerythrocytic merozoites from tissue culture (*2*), irradiated sporozoites from the mosquito (*3*), extracts from blood schizonts (*4*), and emulsified erythrocytic merozoites (*5*). Most studies of these vaccines were made on bird, rodent, and monkey malarias. This report describes the first successful immunization of owl monkeys (*Aotus trivirgatus*) against infection with a human malaria parasite (*Plasmodium falciparum*).

The Uganda–Palo Alto strain (FUP) of

Table 1. Vaccination of *Aotus* monkeys against *Plasmodium falciparum* [Uganda–Palo Alto strain (FUP)] malaria.

Monkey	Composition of vaccine*					Number of infected erythrocytes in the challenge inoculum¶ (day 44)
	KGS† per injection (ml)	FCA‡ per injection (ml)	Parasite protein (mg) in 0.5 ml KGS (injection 1, day 0)§	Parasite protein (mg) in 0.5 ml KGS (injection 2, day 23)‖	Total parasite protein (mg)	
A266	1.0					6.2×10^5
A267	0.5	0.5				6.2×10^5
A268		0.5	1.83	0.9	2.73	6.2×10^5
A269		0.5	1.83	0.9	2.73	6.2×10^5
A270		0.5	1.83	0.9	2.73	6.2×10^5

*Freund's complete adjuvant plus antigen mixed, using a double-hubbed needle and two syringes. Administered intramuscularly in alternate thighs. †Kreb's glucose saline; No. A266 was given two injections of KGS (intramuscularly) on day 0 and on day 23. ‡Freund's complete adjuvant (Baltimore Biological Laboratory, division of Bioquest); No. A267 was given two injections (intramuscularly) of FCA mixed in KGS on day 0 and on day 23. §More than 60 percent segmenters containing individual merozoites. Remainder of the parasite material consisted of schizonts and mature trophozoites. ‖More than 50 percent segmenters containing individual merozoites. Remainder of the parasite material consisted of schizonts and mature trophozoites. ¶Infected blood was obtained from an ongoing FUP infection. Inoculum was given intravenously.

P. falciparum used in this study is being maintained in the laboratory by serial passages of blood-induced infections in owl monkeys (*6*). Modified Harvard medium supplemented with fatty acid–free bovine albumin was used for in vitro cultivation of *P. falciparum* (*7*). Parasitized blood from owl monkeys was cultured in sterile 500-ml sidearm flasks fitted with stoppers with entry ports for a mixture of 95 percent air and 5 percent CO_2. Medium (126 ml) was introduced into each flask and allowed to equilibrate with the gas mixture for 1 hour at 37°C. Then 7 ml of whole heparinized, parasitized blood was introduced into each of the flasks and the gassing was resumed. At the end of a 35- to 40-hour incubation, most of the parasites had developed to mature segmenters containing fully developed individual merozoites. Segmenters were concentrated and merozoites were harvested relatively free of other cellular elements as described previously (*8*). In the final preparation, the antigenic material consisted of 60 to 70 percent merozoites and the remainder, of immature schizonts (Fig. 1).

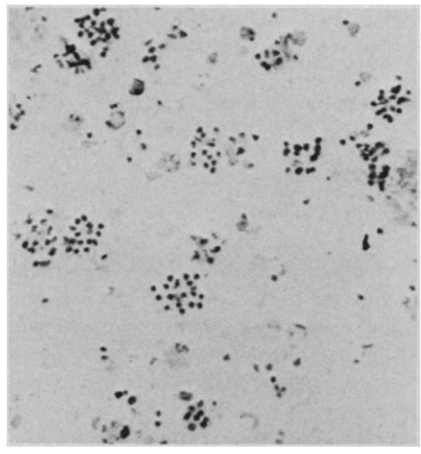

Fig. 1. *Plasmodium falciparum*: segmenters containing fully developed individual merozoites (× 2100).

Five owl monkeys (*Aotus trivirgatus*), weighing approximately 700 g, were used in this pilot experiment. (Composition of the vaccine and the vaccination schedule are summarized in Table 1.) Two monkeys (Nos. A266 and A267) were used as controls; the other three (Nos. A268, A269, and A270) were immunized with *P. falciparum* (FUP strain) merozoites emulsified with equal volumes of Freund's complete adjuvant. This material was administered twice, 3 weeks apart, intramuscularly to each of the three monkeys, with a total of 2.73 mg of parasite protein (1.83 mg on day 0 and 0.9 mg on day 23) being administered to each animal. Immunization never produced a detectable infection. On day 44, that is, 3 weeks after the second vaccination, the five monkeys were challenged by intravenous injection of 6.2×10^5 parasites (FUP strain of *P. falciparum*). Thick or thin blood films (or both) were made daily to follow the course of infection in all monkeys (see Fig. 2). Both control monkeys died within 2 weeks after the challenge: No. A266 died on day 12 with an 81.1 percent infection and No. A267, with a 64.3 percent infection, died on day 13 after challenge. In contrast, the three immunized monkeys survived; No. A269 showed complete protection, while No. A268 became patently infected on day 18 and, after 2 weeks of infection below 0.1 percent, became negative on day 34. However, in No. A270, parasites were first detected on day 11 and the peak parasitemia of 7.0 percent was reached on day 17. By day 30 the parasitemia dropped below 0.1 percent, and since day 34 the animal has been negative. Although the number of monkeys used in this experiment was small, the difference between the course of infection in immunized and nonimmunized animals is indeed very significant, considering the high virulence of *P. falciparum* (FUP) for owl monkeys.

It is concluded from these results that *P. falciparum* merozoite vaccination protects *A. trivirgatus* against homologous infection with intraerythrocytic stages of the normally lethal *P. falciparum* parasites. Two previous attempts to induce immunity in *A. trivirgatus* against this malaria parasite were not very successful (*9*). This is the first report of an immunization experiment with human malaria (using segmenters, cultured in vitro for a short time and containing individual merozoites as antigenic material) in which 100 percent survival has been achieved following a dose of *P. falciparum* lethal to owl monkeys. The same degree of specific immunity has been reported with a nonhuman malaria parasite, *P. knowlesi*, in a rhesus monkey system (*5*). Results reported here are significant with regard to the development of an effective vaccine against human malaria. Through short-term, in vitro cultivation of the blood derived from owl monkeys infected with *P. falciparum* and having high parasitemia, it is possible to obtain potent antigenic material for immunization experiments in these monkeys. The recent landmark discovery that this malaria parasite can be propagated in a continuous culture system in human erythrocytes (*10*) provides significant hope for the availability of merozoites for immunization experiments.

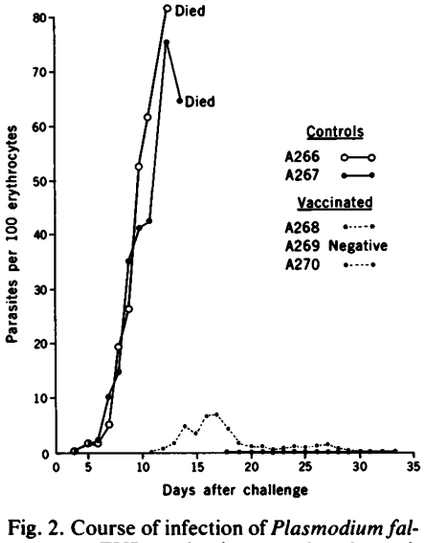

Fig. 2. Course of infection of *Plasmodium falciparum* (FUP strain) in control and vaccinated monkeys (*Aotus trivirgatus*).

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