Malaria Resistance: Artificial Induction with a Partially Purified Plasmodial Fraction

Abstract. Plasmodium berghei *were released from mouse erythrocytes by passage through a French pressure cell. The released organisms were washed and disintegrated; the soluble portion was chromatographed on a Sephadex (G-200) column. The void-volume eluate contained an erythrocyte-free plasmodial fraction which behaved as a vaccine, preventing parasitemia, anemia, and death in mice subsequently challenged with living* Plasmodium berghei.

The isolation of specific, host-free, plasmodial fractions effective in the induction of artificial resistance to malaria has not yet been reported (*1*). Such substances are of crucial importance in advancing the immunochemistry, immunobiology, and vaccine technology of malaria and related diseases (*1–3*). We here report the partial isolation of such a plasmodial fraction.

The A/J mouse model protective system (*4*) was used for detection of resistance-inducing plasmodial fractions. The reticulocyte-infecting *Plasmodium berghei* strain NK65 (line D) served as protective and infective material. This line originated in our laboratories by mosquito passage through hamsters and has been continuously maintained in mice.

Plasmodial materials were prepared as previously reported with modification (*4, 5*). A 20 percent suspension of pooled, washed, parasitized mouse erythrocytes was passed through a French pressure cell at 1000 pounds per square inch (psi; 1 psi = 70.3 g/cm^2). Isotonic phosphate-buffered saline (*p*H 7.0) served as the processing diluent.

The effluent from the pressure cell was freed of unbroken erythrocytes by centrifugation in Kolmer tubes at 1100*g* maximum for 5 minutes each of two times. The hemoglobin supernatant containing "solubilized" erythrocyte and plasmodial components along with free parasites and parasite fragments was centrifuged for 30 minutes at 12,000*g* maximum at 4°C. The sediment containing free parasites and parasite fragments (preparation A) was washed three times and finally resuspended in phosphate-buffered saline (preparation B). In contrast to other methods, plasmodia isolated in this manner have been shown by serologic and ultrastructural examinations to be free from significant amounts of contamination by constituents of the host cell (*5, 6*). The final hemoglobin supernatant (preparation C) resulting from the 12,000*g* centrifugation contained solubilized erythrocyte and plasmodial components.

Resuspended sediment B was disintegrated in the pressure cell at 3000 psi. The effluent was centrifuged at 12,000*g* maximum at 4°C for 30 minutes to remove large nonsolubilized particles (preparation D). The resulting slightly brownish, finely turbid supernatant (preparation E), as well as preparation C, were fractionated through Sephadex G-200 at 4°C, and the optical density at 280 nm was determined for each fraction (Fig. 1).

Fractionation of supernatant C resulted in (i) an initial brownish-red, turbid, void-volume peak C_1, (ii) a clear hemoglobin-containing peak C_2, and (iii) a clear, colorless terminal peak C_3. Fractionation of solubilized isolated plasmodial fraction E resulted in an initial brownish, finely turbid, void-volume peak E_1, and two clear, colorless fractions, E_2 and E_3.

Normal mouse erythrocytes were pooled and processed as above. The resulting preparations were designated as follows: Whole washed erythrocytes (preparation R); erythrocyte sediment following disintegration in the pressure cell and centrifugation (preparation S); and the final hemoglobin supernatant containing "solubilized" erythrocytes (preparation T). The peaks resulting from the fractionation of T on Sephadex G-200 corresponded to those obtained from C above. These were designated T_1, T_2, and T_3, respectively (Fig. 1).

Table 1. Weekly composite mean percentages and ranges of parasitized erythrocytes in protected (PS), unprotected surviving (NPS), and nonsurviving mice (NS).

After challenge (weeks)	Survivors				NS	
	PS		NPS			
	%	Range	%	Range	%	Range
0	0	0 0	0	0 0	0	0 0
1	2.4	0.6– 5.0	3.2	1.6– 6.0	3.1	1.3– 8.8
2	3.2	0– 15.4	14.1	2.6– 28.0	19.0	0.9–40.0
3	0.4	0– 7.9	22.5	2.2– 36.3	28.5	15.0–42.3
4	0.3	0– 4.7	23.1	1.3– 39.0	28.5	12.7–56.0
5	0.1	0– 2.5	26.5	0.7– 43.4	22.9	0.1–41.0
6	<0.1	0– 0.3	13.2	0– 43.0	26.1	23.2–28.9
7	<0.1	0– <0.1	28.4	0– 86.8	69.1	60.5–77.0
8	0.3	0– 9.9	13.4	0– 66.3	71.9	64.8–79.0
9	<0.1	0– 0.4	6.2	0– 68.7	48.7	0–79.1
10	<0.1	0– <0.1	3.8	0– 27.6	15.6	3.3–27.8
11	<0.1	0– 2.2	0.6	0– 9.4	52.8	52.8
12	<0.1	0– 0.7	<0.1	0– <0.1		
14	0	0	<0.1	0– <0.1		

Table 2. Influence of previous treatment on type response and survival of mice following infection challenge.

Previous treatment	Number responding per total*		
	PS	NPS	NS
Plasmodial preparations			
Not fractionated (*11*) A and B, unwashed and washed free plasmodia and fragments C, solubilized infected RBC D, large particle sediment from solubilized B	16/17	0/17	1/17
Sephadex G-200 fractions			
C_1, first peak from C	11/15	1/15	3/15
E_1, first peak from solubilized B	8/10	2/10	0/10
E_2, E_3, and C_2, C_3 fractions after E_1 and C_1, respectively	0/55	5/55	50/55
Normal mouse RBC preparations			
R, washed RBC S, sediment from solubilized RBC T_1, T_2, T_3 Sephadex G-200 fractionation peaks of solubilized RBC	0/44	8/44	36/44

* PS, protected survivors; NPS, unprotected surviving; NS, nonsurvivors.

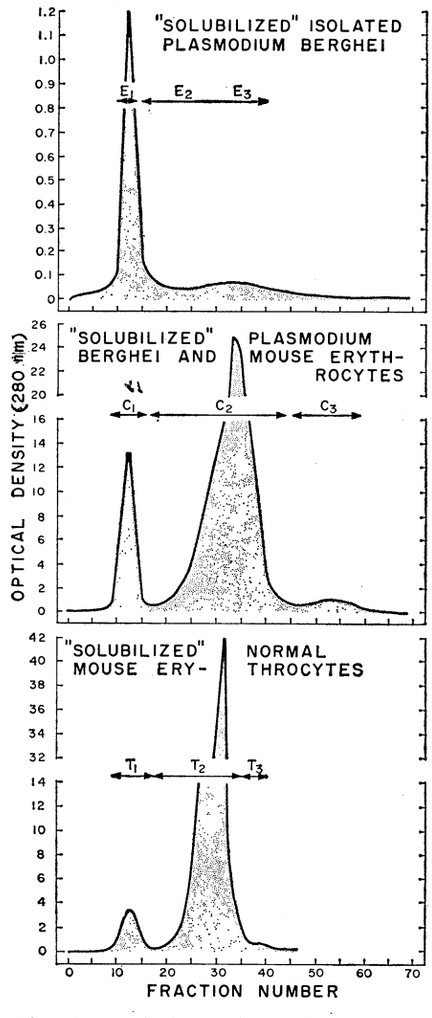

Fig. 1. Optical density (280 nm) of plasmodial fractions. (Top) Free plasmodia and plasmodial fragments; (center) infected mouse red blood cells; and (bottom) normal mouse red blood cells.

Preparations A, B, C, and D and preparations R, S, and T were each injected into a group of mice. Representative fractions from various portions of each Sephadex G-200 peak for each fractionated preparation were pooled, and each pooled sample was injected into its respective group of mice. Each mouse received a single intraperitoneal injection of 1 ml of its respective preparation. All preparations were injected as freshly obtained and proved to be noninfective. All mice were from 6 to 7 weeks of age at the time of injection.

Fifteen weeks later, the animals were challenged intraperitoneally with 10×10^6 mouse cells infected with *P. berghei* NK65D. Three major infection responses were exhibited. The weekly composite mean percentage parasitemias for mice exhibiting each type of response appear in Table 1. Protected mice responded with transient low parasitemias, rapid disease resolution, and high survival rates (PS). Unprotected mice responded with severe protracted parasitemias, a high mortality rate (NS), and a few eventual spontaneous recoveries (NPS). These responses conform to those reported (*4, 7, 8*) for protected and variously treated or nontreated unprotected control A/J mice.

The protective response (PS) was limited to animals receiving crude plasmodial preparation A, B, C, D and partially purified plasmodial fractions C_1 and E_1. Of a total of 42 animals receiving the above materials, 35 demonstrated response PS. Of these, 8 out of 10 receiving plasmodial fraction E_1 exhibited a PS response (Table 2).

None of the 99 mice receiving plasmodial fractions C_2, C_3, E_2, E_3, or any of the normal host cell preparations R, S, T, T_1, T_2, and T_3 demonstrated a protective response. Of the 99 animals, 86 died (response NS) and 13 eventually recovered after a severe infection (response NPS) (Table 2).

The above results confirm the previous finding that the artificial protection against malaria noted here is induced by a particular fraction of plasmodia (*4, 7, 8*) rather than by host cell contaminants. The protective fraction is contained in the partially purified plasmodial material contained in the Sephadex G-200 void volume eluates E_1 and C_1 (*8*).

Although various plasmodial preparations have been evaluated for protective properties (*4, 7–9*), the E_1 fraction prepared in our studies is the most highly purified material thus far shown to induce resistance to malaria. Moreover, in addition to its immunogenic properties, the E_1 fraction has proved an excellent complement-fixing antigen. It is highly specific, sensitive in serological evaluations, and is free from serologically detectable amounts of host erythrocyte stromata (*5, 10*).

Successful demonstration of the protective properties of partially purified plasmodial fraction E_1 opens the way for definitive studies of relations between host and antigen in malaria, as well as final purification and characterization of potential malaria vaccine materials.

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References and Notes

1. A. Zuckerman, *Israel J. Med. Sci.* **5**, 429 (1969); A. Corradetti, *Mil. Med.* **134** (Suppl.), 922 (1969).
2. M. W. Turner and I. A. McGregor, *Clin. Exp. Immunol.* **5**, 1 (1969).
3. E. L. Becker, *Mil. Med.* **131** (Suppl.), **1137** (1966).
4. L. E. D'Antonio, D. T. Spira, P. H. Silverman, *Nature* **223**, 507 (1969).
5. L. E. D'Antonio, A. E. von Doenhoft, Jr., E. H. Fife, Jr., *Proc. Soc. Exp. Biol. Med.* **123**, 30 (1966).
6. R. T. Cook, M. Aikawa, R. C. Rock, W. Little, H. Sprinz, *Mil. Med.* **134** (Suppl.), 866 (1969); V. A. A. Killby and P. H. Silverman, *Amer. J. Trop. Med. Hyg.* **18**, 836 (1969).
7. L. E. D'Antonio, R. C. Fu, D. M. Dagnillo, P. H. Silverman, *J. Protozool.* **16** (Suppl.), abstr. 51, 17 (1969).
8. ———, *ibid.*, abstr. 52, p. 17.
9. G. A. T. Targett and J. D. Fulton, *Exp. Parasitol.* **17**, 180 (1965); A. Zuckerman, J. Hamburger, D. Spira, *ibid.* **24**, 84 (1967).
10. L. E. D'Antonio, A. E. von Doenhoff, Jr., E. H. Fife, Jr., *Mil. Med.* **131** (Suppl.), 1152 (1966).
11. The protection induced by the unfractionated plasmodial materials in this group corresponds to that reported (*4*) for a much larger, similarly treated group in which 35 out of 39 animals showed a protective response.
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Marihuana and Temporal Disintegration

Abstract. *High oral doses of marihuana extract, calibrated for content of* 1(—)-Δ^1-*tetrahydrocannabinol, significantly impaired the serial coordination of cognitive operations during a task that required sequential adjustments in reaching a goal. This disintegration of sequential thought is related to impaired immediate memory.*

Clinical studies (*1*) of temporal distortions and disorganized speech in acute schizophrenia, as well as pilot studies with tetrahydrocannabinol (THC), led us to be interested in temporal disintegration. Temporal disintegration means that the individual has difficulty in retaining, coordinating, and serially indexing those memories, perceptions, and expectations that are relevant to the goal he is pursuing. Our findings indicate that (i) high oral doses