TABLE I. *Chicken spleen cell migration inhibition test with sporozoite and merozoite antigens.*

Chickens		Dilution of antigen					
		Sporozoite			Merozoite		
		1:20	1:40	1:80	1:20	1:40	1:80
Immunized	1	60.60*	75.18	84.10	56.94	79.71	82.03
	2	64.57	71.83	107.18	51.52	66.31	100.80
	3	49.36	87.96	80.45	64.26	71.67	96.15
Control	1	93.35	98.24	106.26	94.37	96.35	113.81
	2	82.18	105.77	104.65	98.87	105.49	98.38

* Migration index.

tion and then minced into 1-mm^3 fragments. Five fragments of spleen were placed in a petri dish containing fresh chicken plasma. Several minutes later, the excess plasma was aspirated away and then the culture medium with or without antigen was added. On day 4 after cultivation, cellular migration from the edge of the fragment was measured by an ocular micrometer. Mean migration distance was calculated from the migration distances of 10 fragments. Migration index was calculated by the following formula:

$$\text{Migration Index} = \frac{\text{Mean migration distance in the presence of antigen}}{\text{Mean migration distance without antigen}} \times 100.$$

As shown in Table I, the migration of splenic cells from infected chickens was inhibited remarkably with each antigen at the dilution of 1:20, but no migration inhibition was observed at a dilution of 1:80. The degree of migration inhibition was increased concomitantly with the increase of antigens.

It is concluded that cell-mediated immunity for *E. tenella* infection in chickens is detectable by the splenic cell migration inhibition test.

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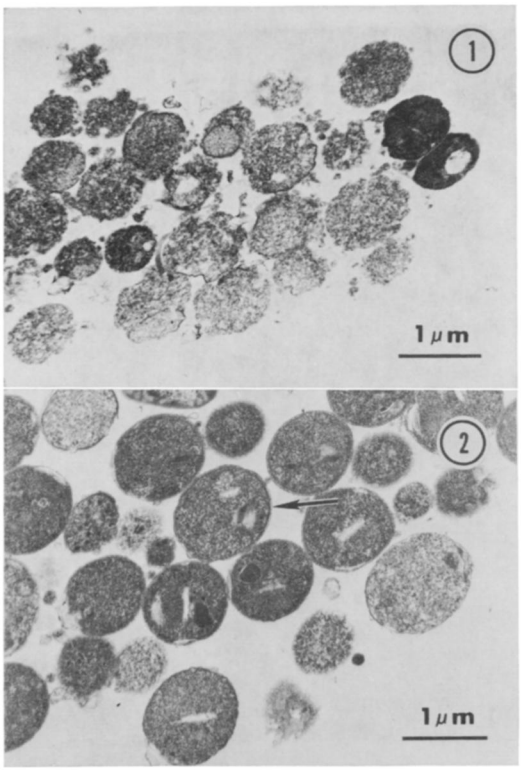
A Comparison by Electron Microscopy of *Plasmodium berghei* Freed by Ammonium Chloride Lysis to *P. berghei* Freed by Ultrasound in a Continuous-flow System

One of the most frustrating problems in studying the immunology and biochemistry of malaria parasites has been our inability to obtain intact parasites free of host cell contamination. Cook et al. (1969, Mil. Med. **134:** 866–883) evaluated the then existing procedures for freeing malaria parasites and concluded that while none were completely satisfactory the French pressure cell technique of D'Antonio et al. (1966, Proc. Soc. Exp. Biol. Med. **123:** 30–34) was probably the best. Recently two novel procedures, one using ammonium chloride lysis, and one ultrasound have been reported.

In the present note, *Plasmodium berghei* freed from their host erythrocytes by ammonium chloride lysis by the procedure described by Martin, Finerty, and Rosenthal (1971, Nature **233:** 260–261) and by treatment in a continuous-flow ultrasonic system (Prior and Kreier, 1972, Exp. Parasit. **32:** 239–243) were examined for morphological integrity with the electron microscope. The free parasites were prepared for electron microscopy according to the procedures described by Cook et al. (1969, Mil. Med. **134:** 866–883) and thin sections were examined using a Zeiss EM 9 S electron microscope.

Figure 1 is an electron micrograph of *P. berghei* released from their host erythrocytes by ammonium chloride lysis. The parasites are free of entrapping host cell membranes.



FIGURES 1, 2. Electron micrographs of *Plasmodium berghei*, freed from host cells. **1.** By ammonium chloride lysis. The cells are damaged. **2.** By sonication in a continuous-flow system. The pellicular complex (arrow) and cytoplasm are intact.

However, the parasites appear to be damaged since the cytoplasm is leached out of most of the parasites. Neither the internal fine structure nor the pellicular complex are well preserved.

The electron micrograph shown by Martin et al. in their paper is of parasites in somewhat better condition than those we prepared by ammonium chloride lysis; however, it also shows structures which may be host cell membranes and partially lysed erythrocytes. It is probable that the difference between the time required to release parasites by ammonium chloride lysis and that at which the parasites in turn are lysed is very narrow and that we exceeded it in our effort to eliminate host membrane.

Figure 2 is an electron micrograph of *P. berghei* released from their host erythrocytes by continuous-flow sonication. The parasites are free from the erythrocytes and no red cell membranes remain. The multilayered pellicular complex (arrow) is preserved as is the internal structure of the ultrasonically freed parasites. No leaching of the cytoplasm from the parasites occurred during the sonication.

Both procedures freed malarial parasites from their host erythrocytes. Yields of free parasites were slightly higher with ammonium chloride lysis than with the sonic oscillation technique since by the former procedure all erythrocytes were lysed. However, considerable damage to the parasites occurred during ammonium chloride lysis, probably by chemical alteration of the parasites' outer membranes.

It is probable that by the ammonium chloride technique it is difficult to eliminate all the host membrane and not damage the parasites in the process. The parasites freed by ultrasound were morphologically intact. This is apparently due to the fact that the mechanical action of the ultrasound affects mainly the outer surfaces of the erythrocytes during their passage through the sound field and due to the adjustment of the sample flow rate so that the free parasites leave the ultrasonic field as they are released from the erythrocytes. An advantage of the ultrasound technique for freeing plasmodia over other available techniques resides in its easy operation and uncritical nature.

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