The time of appearance of depth perception and independent locomotion in normal infant monkeys confirms that reported by others (*7*). We believe ours to be the first report of development of these three types of behavior in monkeys subjected to birth asphyxia. Although these functions were delayed in the asphyxiated monkeys, there was little difference from the normal monkeys in the effectiveness with which the responses were made after they had become established.

Previous investigators have suggested (*6*) that visual depth perception is adaptive and that one of the keys to survival of a species is development of visual depth perception by the time locomotion becomes independent. Such an ability appears to be innate and does not seem to depend on learning during early life. Evidence for this was found in the time of onset of depth perception in other animals: the chick and goat, 1 day; the rat and cat, 3 or 4 weeks; the human infant, 6 to 10 months (*6, 7*). According to our own observations, the onset of these two responses is nearly coincident with the development of visual placing.

The distribution of focal lesions in rhesus monkeys subjected to asphyxia at birth was found to involve afferent systems, such as somethetic, auditory, and vestibular, but the visual pathways were spared (*1*). Although the brains of the still living monkeys of the present study will not be examined for some time, we assume that they have been injured and that the pattern of damage is the same as that in other animals subjected to birth asphyxia for a comparable period of time. In spite of this presumed brain damage, these developmental behaviors were fully expressed in the asphyxiated monkeys, although significantly later than in nonasphyxiated monkeys.

Monkeys surviving neonatal asphyxia after 8 to 10 years showed severe memory deficits for events in the immediate past (*8*). Short-term memory deficits also occurred within the same time limits in monkeys at 10 months after birth asphyxia (*9*). In addition, Saxon and Ponce (*10*) found evidence of performance deficits of asphyxiated monkeys at 6 to 7 months of age; these were observed in learning set problems, delayed response, and perseveration tasks, all of which depend upon visual stimuli. When compared with the establishment of these developmental behaviors, deficits in learning and memory (*10, 11*) (although significantly delayed) suggest that brain damage by neonatal asphyxia can result in a degree of dissociation of developmental and acquired behavior. Whatever organic basis there may be to explain our findings remains to be discovered. Nevertheless, the late appearance of developmental behaviors may be one of the first observable signs of brain damage and mental retardation. Rather than regarding these events as transient and negligible, they should be systematically evaluated in the human infant with a view toward early diagnosis.

JERI A. SECHZER

Edward W. Bourne Behavioral Laboratory, New York Hospital–Cornell Medical Center, Department of Psychiatry, Westchester Division, White Plains, New York 10605

MARIA D. FARO, JUNE N. BARKER
DAVID BARSKY, SERGIO GUTIERREZ
WILLIAM F. WINDLE

Institute of Rehabilitation Medicine, New York University Medical Center, New York 10016

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Malaria Resistance: Artificial Induction with a Partially Purified Plasmodial Fraction

D'Antonio *et al.* (*1*) have described a fraction of *Plasmodium berghei* that confers a measure of protection against subsequent challenge by the organism. However, we now call attention to an impression given by their paper which we regard as misleading. They state: "In contrast to other methods, plasmodia isolated in this manner [French pressure cell] have been shown by serologic and ultrastructural examinations to be free from significant amounts of contamination by constituents of the host cell." They then refer to an article by us (*2*) and one by Killby and Silverman (*3*), implying that these articles support their statement on ultrastructural grounds. In point of fact, neither article supports their contention. For example, in the article by Killby and Silverman, figure 5 shows two parasites surrounded by large amounts of cell debris, including many membrane vesicles. Our results [figure 2b in (*2*)] showed the same thing—that is, the complete fragmentation of most parasites by French pressure cell lysis of the parasitized erythrocytes. It is clear that many of the membrane vesicles in these micrographs originated from the host erythrocyte since they are not "solubilized." Otherwise, D'Antonio *et al.* would not have been able to prepare normal "erythrocyte sediment" by the same technique.

D'Antonio *et al.* have lowered the initial lysis pressure from that used to obtain the preparations examined by Killby and Silverman and by us. However, in the absence of published low power electron micrographs showing both (i) many intact parasites and (ii)

the absence of host cell membranes, we must reserve judgment. Both criteria are necessary since once the parasites are ruptured, they contribute smooth membrane vesicles which are morphologically indistinguishable from erythrocyte vesicles produced by a shearing process such as French pressure cell lysis.

We are not denying that plasmodial antigens can be isolated from preparations such as that described by D'Antonio *et al.* However, their description of centrifugations and washing procedures after lysis of the parasites makes it difficult, if not impossible, to judge whether their antigen preparation is soluble or particulate. They describe centrifugation of the pressure cell effluent at 1100*g* maximum for 5 minutes twice in succession. The pellets from these centrifugations are apparently discarded, yet they undoubtedly contain the great majority of whatever unbroken parasites came through the French pressure cell. We have demonstrated (*2*) that saponin-lysed preparations of *P. knowlesi* sediment completely at 370*g* (average) in 10 minutes. We have no reason to believe that *P. berghei* is substantially different. This indicates to us that D'Antonio *et al.* have as starting material whatever soluble components of the parasites were released upon initial pressure cell treatment plus intracellular organelles such as ribosomes, plus small membrane vesicles of both parasite and host erythrocytes which do not all sediment at low gravitational fields.

This difficulty might be very simply overcome by the use of a generally accepted operational definition of solubility, such as lack of significant sedimentation at 105,000*g* (average) for at least 1 hour. If their antigen preparation is soluble by this criterion, the task of purifying and characterizing it will be greatly simplified, and the problems of particulate host cell contaminants for soluble malarial antigen preparations will have been lessened.

ROBERT T. COOK
MASAMICHI AIKAWA
Institute of Pathology, Case Western Reserve University, Cleveland, Ohio

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After examining a large number of plasmodia released by the French pressure cell in a series of preparations, Killby and Silverman (*1*) concluded, "Cell disintegration by French press released the largest proportion of structurally intact parasites generally lacking closely associated arrays of erythrocyte membranes."

Cook *et al.* (*2*) apparently studied only one French pressure cell (FPC) preparation of plasmodia. They did not comment on the presence or absence of host erythrocyte materials in this preparation, though they clearly identified host erythrocyte materials in their plasmodial preparations obtained by other methods. By failing to make a clear distinction between their identification of host materials in their other preparations and their inability to make such identification in their FPC-derived plasmodial preparation, they unwittingly invited the impression that such contamination was also to be identified in the FPC preparation.

Cook *et al.* (*2*) noted in the FPC preparation, as did Killby and Silverman (*1*), occasionally preserved parasites and a generally distributed cellular debris. They concluded, both in (*2*) and in their statement above, that the fragmented appearance of the FPC-derived plasmodial materials was directly due to the effects of the FPC passage itself. In fact, neither their studies (*2*) nor Killby and Silverman's (*1*) can be considered definitive quantitative or ultrastructural assessments of the parasites themselves immediately after FPC release. Both groups studied the FPC parasite materials only after they were subjected to vigorous wash procedures. Unlike the stroma-encircled saponin-lysed preparations mentioned by Cook *et al.*, the stroma-free parasites released by FPC are potentially more vulnerable to the disruptive effects of vigorous washing. The large mature forms of the parasite may be especially fragile.

There is convincing evidence that much of the cellular debris in the washed FPC plasmodial sediment of Cook *et al.* (*2*) and Killby and Silverman (*1*) is of parasite origin. Thus, centrifugation of FPC-passed (1000 psi; 1 psi = 70.3 g/cm^2) normal red blood cells, at 12,000*g* maximum for 30 minutes, results in no more than a thin film sediment. This sediment, to which Cook *et al.* allude, would constitute only a small fraction of the bulky parasite sediment obtained by similar centrifugation of the similarly FPC-passed plasmodially infected blood. In addition, in *Plasmodium knowlesi* sediments obtained in a manner identical (*3*) to that of Cook *et al.* (*2*) only trace amounts of host stroma were detected by the complement fixation test (*3*). The partially purified antigens derived from these sediments were serologically free of host stromal contamination (*3*).

Cook *et al.* relate the sedimentation properties of saponin-treated erythrocytes infected with *P. knowlesi* to that of our FPC-released *P. berghei* parasites (*4*). Not only do the FPC-released *P. berghei* parasites differ in that they are free of encircling stroma, but also in that they are made up of many parasites from small trophozoites to large segmented forms and should sediment in a pattern much different from that of the uniformly fully mature *P. knowlesi*.

Discussion of the physical form of the parasite antigens should be reserved until their actual physical character is established. We chose to use the term "solubilized" (*4*) in quotation marks to indicate a noncommitment. Perhaps we might have used the term "disintegrated."

In terms of the final, active, partially purified *Plasmodium berghei* antigens obtained by us (*4*), the losses encountered by centrifuging the immediately obtained FPC effluent at 1100*g* to remove the few remaining undisintegrated red blood cells was secondary. Though more efficient means can undoubtedly be devised, these initial losses were acceptable in assuring ourselves of virtual removal of any residual intact red blood cells. Many of the smaller, free *P. berghei* forms remained behind with the large parasite fragments; both were later sedimented at 12,000*g* maximum for preparation of the partially purified *P. berghei* antigen fraction.

LAWRENCE E. D'ANTONIO
PAUL H. SILVERMAN
Department of Zoology, University of Illinois, Urbana 61801

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