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INTRAERYTHROCYTIC PLASMODIAL CALCIUM METABOLISM

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SUMMARY

Both prokaryotes and eukaryotes require Ca^{++} for a variety of cellular functions. Intraerythrocytic plasmodia, however, exist within a cell ordinarily impermeable to external Ca^{++}. Our investigations of Ca^{++} homeostasis in murine *Plasmodium berghei* reveal that (1) infected erythrocytes contain 10 - 15 times as much Ca^{++} as do uninfected red cells, (2) these large amounts of Ca^{++} are located almost exclusively within the parasite, and (3) the parasite obtains at least a portion of this Ca^{++} through causing increased permeability of the host cell membrane to external Ca^{++}.

INTRODUCTION

Intracellular parasites such as plasmodia exist in an environment rich in many nutrients and high energy organophosphate compounds. However, some substances readily available extracellularly may be scarce within the cytosol of the host cell. Plasmodia may have mitochondria (1), actively phagocytose elements of the host cell cytoplasm (2), replicate, and, at least in extracellular form, are motile (3). These organelles and processes require Ca^{++}, an element which is almost absent within the normal erythrocyte. Not only are mammalian red cells almost impermeable to Ca^{++} (4,5), but what little does leak into the cell is actively extruded by Ca^{++}ATPase. To determine whether intraerythrocytic plasmodia might obtain Ca^{++} despite the barrier imposed by the host cell membrane, we have investigated Ca^{++} metabolism within *P. berghei*-infected murine erythrocytes.

METHODS

In these investigations, we employed Swiss White mice infected with *P. berghei* (NYU-2 strain). The infection was maintained and passed as previously described (6). Infected mice were ether anesthetized, and heparinized blood was collected following axillary incision. White cells and platelets were removed by filtration of the blood through powdered cellulose (7).

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Table 1. Ca^{++} content of normal and *P. berghei*-infected mouse red cells and of isolated *P. berghei*. Centrifugal separation of pooled red cells from 4 - 6 animals was accomplished by centrifugation at low speed (~2,000 x g) for 15 minutes in an angle-head rotor at 23°C. Top, middle and bottom fractions were assayed for Ca^{++} as detailed in Methods. Some loss of intraerythrocytic Ca^{++} appears to occur under these conditions.

SAMPLE	n*	CALCIUM CONTENT (μM/l packed cells)
Normal RBC	19	14 (± 5)
Infected RBC (avg parasitemia of all determinations = 60%)**	22	201 (± 71)***
Centrifugally separated infected RBC		
A. Top (88 ± 20% infected)	9	208 (± 70)***
B. Middle (54 ± 27% infected)	9	110 (± 44)***
C. Bottom (23 ± 12% infected)	9	50 (± 23)***
Isolated parasites	6	449 (±182)

* number of samples independently prepared and analyzed.
** range = 50-87
*** significantly different from values for uninfected erythrocytes at $p < 0.001$ (Student's "t" test, two-tailed).

For absolute Ca^{++} analyses, the red cells were washed quickly four times in Ca^{++}-free, cold isotonic NaCl. In experiments employing isolated parasites, host cells were selectively lysed by suspension in ammonium chloride as previously described (8). Extraction of cells or parasites with 10% trichloroacetic acid (containing 10,000 ppm lanthanum) and atomic absorption spectroscopy (nitrous oxide/acetylene flame) were performed as reported earlier (4). Protein was measured by the technique of Lowry (9) and packed cell volumes were determined by microhematocrit.

The uptake of $^{45}Ca^{++}$ by normal and *P. berghei*-infected mouse erythrocytes was determined in erythrocytes suspended to hematocrit 5 volumes % in Hanks balanced salt solution (containing 1.25×10^{-3}M $CaCl_2$) with 0.1 μCi/ml $^{45}Ca^{++}$. The cells were incubated one hour at 37°C and then rapidly washed thrice in cold isotonic NaCl. Cell-associated $^{45}Ca^{++}$ was measured by scintillation counting of deproteinized 10% trichloroacetic acid extracts as previously reported (4). Ca^{++} uptake was calculated using the known specific activity of external $^{45}Ca^{++}$, with appropriate corrections for incomplete packing of the washed erythrocytes.

RESULTS AND DISCUSSION

The Ca^{++} content of erythrocytes from mice infected with *P. berghei* is almost 15 times that of red cells from uninfected animals (Table 1). This increased Ca^{++} is largely or completely within parasitized red cells as indicated by the fact that erythrocyte Ca^{++} is linearly related to the severity

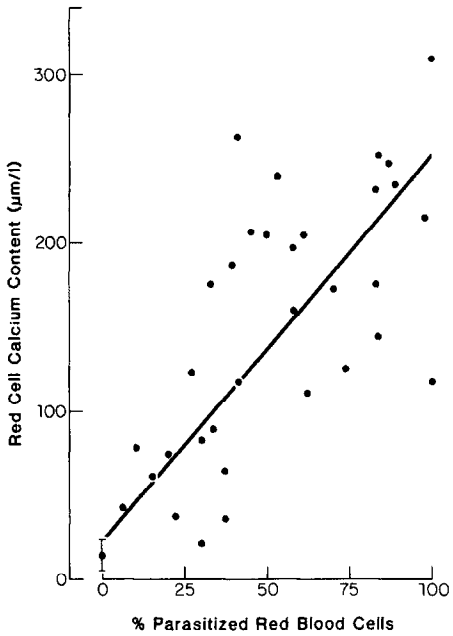

Figure 1. Ca^{++} content of normal and *P. berghei*-infected mouse erythrocytes. Vertical bar on left represents the range of values for 19 normal mice, and points are individual values from 33 infected animals. Correlation coefficient = +0.857, $p < 0.01$. The solid line represents the calculated linear regression of Y on X ($y = 2.27x + 23.53$).

of infection (Fig. 1). Furthermore, when red cells from infected animals are separated by low-speed density centrifugation, the most dense cell populations (primarily uninfected erythrocytes) have near-normal Ca^{++} content (Table 1). Therefore, the increased red cell Ca^{++} is probably characteristic only of infected cells and does not reflect secondarily disordered Ca^{++} homeostasis in uninfected red cells. The large amounts of Ca^{++} in infected red cells are probably within the parasite itself, rather than the cytoplasm or membrane of the host erythrocyte. When we measured the absolute Ca^{++} content of parasites isolated by ammonium chloride lysis, we found Ca^{++} levels in parasites greater than 30 times those of normal murine red cells (Table 1).

To determine how the parasite might obtain this Ca^{++}, we measured the rate of influx of external, isotopically-labelled $^{45}Ca^{++}$. As shown in Figure 2, the rate of Ca^{++} influx into infected red cells is quite brisk, at

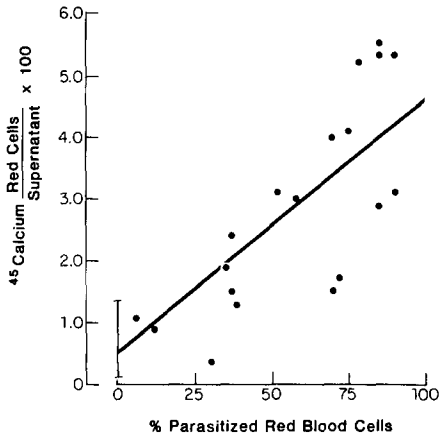

Figure 2. Uptake of $^{45}Ca^{++}$ by normal (vertical bar) and *P. berghei*-infected (individual points) mouse erythrocytes. Values shown are derived from 9 normal and 19 infected animals. Note that the accumulation of $^{45}Ca^{++}$ necessarily reflects both the exchange of external (isotopic) $^{45}Ca^{++}$ for internal Ca^{++} and the net accumulation of $^{45}Ca^{++}$. Correlation coefficient = +0.852, $p < 0.01$. The solid line represents the calculated linear regression of Y on X ($y = 0.041x + 0.052$).

least 7 times that which occurs in normal red cells. This enhanced rate of Ca^{++} influx is correlated with the severity of malaria infection (Figure 2).

Similar Ca^{++} accumulation within normal mouse red cells will cause drastic shape changes, metabolic depletion, and potassium and water loss as we have earlier described in Ca^{++}-loaded human red cells (10). Because malaria-infected red cells do not display similar abnormalities, the Ca^{++} which does enter infected erythrocytes must be efficiently concentrated by the malaria parasite, rather than accumulated in the host cell.

These results serve to emphasize an aspect of the metabolism of intracellular parasites which has attracted little attention. That is, although intracellular parasites may enjoy many nutritional advantages over free-living organisms, the availability of some nutrients may be greatly restricted by permeability characteristics and metabolic processes of the host cell. Therefore, intracellular parasites must have special provisions for obtaining and concentrating these substances. In this particular case, we have shown that the intraerythrocytic form of *P. berghei*, although it exists in

the Ca^{++}-poor cytosol of the erythrocyte, maintains high internal concentrations of Ca^{++} through modification of the permeability of the host cell membrane. Knowledge of such special facilities and requirements of intracellular parasites like plasmodia eventually may lead to alternative approaches to therapy.

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