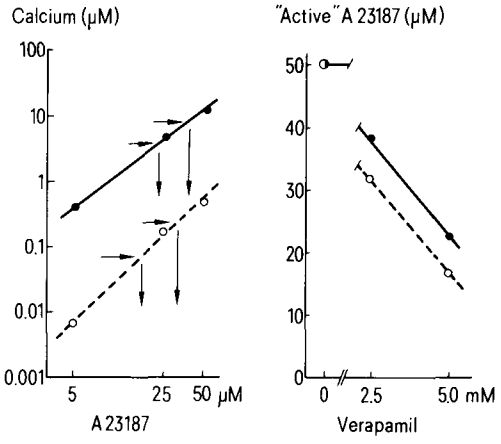
0.34 and 35.3 nM. The difference between these 2 values reflects the dose-related increase in calcium translocation at increasing calcium concentrations, saturation of the ionophore being only observed at much higher calcium concentrations in the initial aqueous phase (15–20 mM; data not shown). Factor b decreased from 1.892 ± 0.038 to $1.509 + 0.014$ as the calcium concentration was raised from 8 µM to 1.0 mM. This behaviour is due to the fact that each atom of calcium is complexed by 2 molecules of ionophore[4] so that factor b fluctuates between the extreme values of 2 (theoretical value in the presence of infinitely low calcium concentrations) and 1 (experimental value seen at saturating calcium concentrations)[5]. The exponential relationship illustrated in the figure (left panel) permitted the conversion of the amount of calcium translocated in the presence of both A23187 and verpamil into the corresponding 'active' concentration of ionophore, i.e. the concentration of A23187 which would translocate the same amount of calcium if the experiment had been performed in the absence of verapamil. When the 'active' concentrations of ionophore were plotted as a function of the verapamil concentration, it became obvious that calcium antagonizes the effect of verapamil in inhibiting calcium translocation. Under the present experimental conditions, the protective effect of calcium corresponded to a shift to the right of the dose-action response to increasing concentrations of verapamil (figure, right panel), suggesting a competitive type of antagonism. In further experiments, a series of lines parallel to those shown in the right panel of the figure were obtained when the calcium concentration was further increased to 4.0 and 20.0 mM. Control experiments indicated that the protective effect of calcium could not be attributed to any direct interference of the cation with verapamil itself.



Left panel: effect of increasing concentrations of A23187 on calcium translocation (logarithmic scales); the arrows refer to the values seen in the presence of both A23187 (50 µM) and verapamil (2.5 and 5.0 mM), and illustrate the mode of calculation for the 'active' concentration of ionophore. Right panel: effect of increasing concentrations of verapamil (logarithmic scale) upon the 'active' concentration of ionophore. The concentration of calcium in the initial aqueous phase amounted to 8.0 µM (open circles, dotted lines) or 1.0 mM (closed circles, solid lines).

The pattern of parallel lines illustrated in the right panel of the figure is superimposable on that characterizing the protective effect of calcium against the inhibitory action of verapamil upon physiological processes, such as glucose-induced insulin release[6]. This analogy reinforces the view that the modality by which verapamil affects A23187-mediated calcium translocation in the present model may be relevant to the mode of action of the organic calcium-atagonist in biological systems.

1 This work was supported by grants from the Belgian Foundation for Scientific Medical Research.

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Mean cytoplasmic protein concentration of host erythrocytes and the reticulocyte response in *Plasmodium berghei* infected mice

M.J. Autuori

Biology Department, University of Bridgeport, Bridgeport (Conn. 06602, USA), 23 February 1979

Summary. The decreasing mean cytoplasmic protein concentration (MPC) of *P. berghei* host cells is paralled by an increasing parasitemia and percent reticulocyte response. The reticulocyte response parallels the percent parasitemia except during a period of accelerated reticulocytosis noted during the midpoint of the infection at which time the percent reticulocytes increases at a rate more than double the rate of increase of percent parasitemia. Although the reticulocyte population and the host cell population are usually equivalent, the disparity noted suggests the existence of unique characteristics in the reticulocyte response of *P. berghei* infected mice.

The mean cytoplasmic protein concentration (MPC) of *Plasmodium berghei* host erythrocytes has been measured in mice during patent infections[1]. The decrease in host cell MPC throughout the infection is inversely related to the percent of parasitized erythrocytes. *P. berghei* preferentially invades reticulocytes[2–4]. This study explores the relationship which exists between host cell MPC, the reticulocyte response and the percent parasitemia of the infected animal. In erythrocytes, MPC is essentially equivalent to hemoglobin concentration (%w/v), hemoglobin comprising from 95%[5] to 97%[6] of the erythrocyte dry weight.

Materials and methods. The KBG 173 strain of *Plasmodium berghei* and young female virgin Swiss mice weighing between 15 and 25 g were used in this investigation. An average of 5×10^5 parasites, suspended in 0.825% saline, was inoculated i.p. into each experimental animal with

dosage adjusted to animal weight. Blood smears for parasitemia determination were stained with Wright's stain. Percent parasitemia was computed as the number of parasitized erythrocytes per thousand erythrocytes. Blood smears for reticulocyte counts were stained with new methylene blue. Percent reticulocytes was computed as the number of reticulocytes per thousand erythrocytes, but no attempt was made to separate reticulocytes according to age[3]. The degree of anemia was measured by determining the number of erythrocytes per mm^3 using an improved Neubauer hemocytometer. Stock albumin solution (32% w/v hemoglobin equivalent) were prepared by dissolving bovine plasma albumin Cohn fraction V in 0.7% saline while monitoring the solution with a hand refractometer calibrated with a hemoglobin standard. Dilutions of the stock solution were made with 0.825% saline. Refractometry was performed according to established methods[1,5,7,9].

Results. Host cell MPC drops from an initial 27.4% to a final 20.1%. This drop is significant at the 0.001% level (standard Student's t-test). The MPC drop from day 1 to day 3 is not statistically significant, nor is the MPC drop from day 5 to day 9. However, the MPC drop from day 3 to day 5 is significant at the 0.05% level. The percent parasitemia rises from an initial 1.9% on the 1st patent day to a final 22.7%, while the percent reticulocytes rises from 1.4% on day 1 to 24.5% on day 9. The number of erythrocytes/mm^3 drops from 9.73×10^6 on day 1 to 1.79×10^6 on day 9.

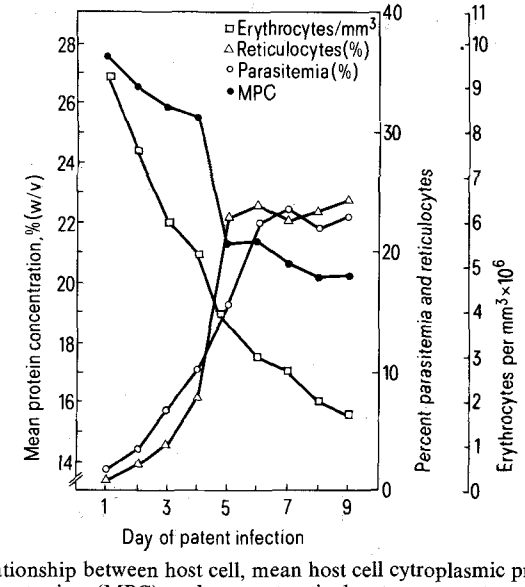
Discussion. MPC and percent parasitemia values in this investigation closely correspond to those reported previously[1]. There is a relatively inverse relationship between host cell MPC and percent parasitemia curves, but the percent reticulocyte curve is almost the mirror image of the host cell MPC curve if allowance is made for the different ordinate scales. The curves exhibit a gradual change from day 1 to day 3, a marked change from day 4 to day 5 and a relatively level region or plateau for the remaining days.

Visual comparison of the percent parasitemia and percent reticulocytes curves reveals a very close correspondence on all days except day 5 when the increase in percent reticulocytes is more than twice that of percent parasitized host cells. It appears that from day 4 to 5 reticulocytes enter the peripheral blood at an accellerated rate. The influx of reticulocytes during this period outpaces the ability of the parasites to invade them. By day 6 however, the parasites have successfully invaded the expanded reticulocyte population and the percent parasitemia and percent reticulocytes curves assume the relatively unchanging values characteristic of the plateau phase which persists until death.

Other investigators[10] have noted a similar phenomenon in the reticulocyte response of *P. berghei* infected rats immediately preceding the period of parasite decline, and have advanced an hypothesis suggesting that the reticulocytes produced during this 'penultimate period' do not support parasite development. This may be true in the rat because rats often recover from infection after crisis. In mice, the rapid influx of reticulocytes provides an expanded host cell population for the parasites, albeit after a lag period of approximately 24 h (day 5 to day 6). It is important to note that the MPC of the expanded reticulocyte population in mice (days 5 to 9) is significantly lower than the MPC of reticulocytes early in the infection. The full impact of this change in the intracellular environment remains to be elucidated.

P. berghei infections in mice are characterized by an initial parasitemia rise followed by a plateau level of infection which persists until death[11]. Not only do *P. berghei* parasites show a marked preference for reticulocytes[2,3], but the infection can be completely blocked when reticulocyte production is repressed[12]. It has been shown that certain *P. berghei* parasites, which have invaded mature erythrocytes, degenerate and are extruded from these cells[13]. These facts and the close correspondence between the parasitemia and reticulocyte curves depicted in the figure suggest that, during the plateau phase of *P. berghei* infection in mice, the reticulocyte population is effectively equivalent to the host erythrocyte population. The high MPC values noted prior to day 5 may be at least partially atributed to the invasion of mature erythrocytes. Visual evidence of this phenomenon has been obtained during both MPC and percent reticulocytes determinations.

The percent reticulocytes curve exhibits a plateau from day 5 to 9, but the number of erythrocytes/mm^3 on day 9 is less than half the number on day 5. Preliminary experiments in this laboratory reveal no plateau in the percent reticulocyte response of uninfected mice made anemic by repeated bleeding. The percent reticulocyte response in these animals is inversely proportional to the erythrocyte count. These findings further suggest the uniqueness of the reticulocyte response in *P. berghei* infection and generate some fascinating questions which merit further investigation.



Relationship between host cell, mean host cell cytroplasmic protein concentration (MPC) and percent reticulocytes, percent parasitemia and erythrocytes/mm^3.

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