Transport and Metabolism of the Essential Vitamin Pantothenic Acid in Human Erythrocytes Infected with the Malaria Parasite Plasmodium falciparum*

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The growth of the human malaria parasite, Plasmodium falciparum, within its host erythrocyte is reliant on the uptake of a number of essential nutrients from the extracellular medium. One of these is pantothenic acid, a water-soluble vitamin that is a precursor of coenzyme A. In this study we show that normal uninfected erythrocytes are impermeable to pantothenate but that the vitamin is taken up rapidly into malaria-infected cells via a transport pathway that has the characteristics (furosemide sensitivity, nonsaturability) of previously characterized, broad specificity permeation pathways induced by the intracellular parasite in the host cell membrane. The transport of pantothenate therefore constitutes a critical physiological role for these pathways. Inside the parasitized cell pantothenate undergoes phosphorylation, the first step in its conversion to coenzyme A. Parasites within saponin-permeabilized erythrocytes were shown to take up and phosphorylate pantothenate, consistent with the intracellular parasite having both a pantothenate transporter and a pantothenate kinase. Comparisons of the rate of phosphorylation of pantothenate by lysates prepared from uninfected and infected erythrocytes revealed that the pantothenate kinase activity of the P. falciparum trophozoite is some 10-fold higher than that of its host cell and that most, if not all, of the phosphorylation of pantothenate within the malaria-infected cell occurs within the intracellular parasite. These results contrast with those of previous studies in which it was proposed that the avian malaria parasite Plasmodium lophurae lacks pantothenate kinase (as well as the other enzymes for the synthesis of coenzyme A) and is reliant upon the uptake of preformed coenzyme A from the host cell cytosol.

Malaria is caused by a protozoan parasite (genus Plasmodium) which, during the course of its life cycle, invades the red blood cells of its vertebrate host. This strategy serves to protect the parasite from attack by the immune system of the host; however, it poses significant challenges to the protozoan with regard to obtaining essential nutrients from the plasma.

One such compound is pantothenic acid, a water-soluble vitamin (M₉ 219; Fig. 1) that serves as a precursor of the enzyme cofactor coenzyme A. Early evidence for the importance of exogenous pantothenate in supporting the growth of the intracellular malaria parasite came from the observation that in chickens infected with the avian parasite Plasmodium gallinaceum a dietary deficiency of pantothenate resulted in a marked reduction in the parasitemia (1). Forty years later it was demonstrated in in vitro growth assays that the sustained growth of the human malaria parasite Plasmodium falciparum within human erythrocytes is dependent upon there being pantothenate in the extracellular medium (2). The requirement of the intracellular malaria parasite for an exogenous supply of pantothenate implies that there is a mechanism by which this compound can gain entry into the infected erythrocyte. However, this process has not been characterized.

In mammalian tissues, as well as in bacteria, pantothenate is converted to coenzyme A via a series of enzyme-mediated steps, the first of which involves the phosphorylation of pantothenate by pantothenate kinase (3, 4). In a series of studies with Plasmodium lophurae-infected duck erythrocytes Trager and colleagues (5, 6) obtained evidence that all five enzymes involved in the conversion of pantothenic acid to coenzyme A are present in the erythrocyte cytosol of both parasitized and nonparasitized duck red cells but absent from the intracellular parasite. This led to the proposal that in malaria-infected erythrocytes pantothenic acid is converted to coenzyme A within the host cell cytosol, from which it is taken up by the intracellular parasite. Support for this model came from the earlier observation that the survival of erythrocytic stages of P. lophurae removed from their host red cells is favored by the inclusion of coenzyme A (but not by the inclusion of pantothenate) in the extracellular medium (7, 8). It remains to be established, however, whether the same model is applicable in the case of P. falciparum-infected human erythrocytes.

In this study we have investigated the transport and subsequent phosphorylation of pantothenate in human erythrocytes infected in vitro with P. falciparum. Our results indicate that the mature human erythrocyte lacks a functional pantothenate transporter but that the vitamin enters malaria-infected erythrocytes rapidly via the broad specificity, new permeation pathways (NPP)¹ induced by the parasite in the infected cell membrane (9–13). Once inside the parasitized cell, pantothenate undergoes phosphorylation. Data from experiments carried out with saponin-permeabilized parasitized erythrocytes and with cell lysates and extracts indicate that the intraerythrocytic form of P. falciparum has the ability to take up and metabolize

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¹ The abbreviations used are: NPP, new permeation pathways; PCA, perchloric acid; fl, femtoliters; ATP·S, adenosine 5′-O-thiophosphate.
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Pantothenate and that most, if not all, of the phosphorylation of pantothenate within the parasitized erythrocyte occurs within the intracellular parasite. The model proposed previously for *P. falciparum*-infected avian erythrocytes in which the parasite is wholly reliant upon a supply of preformed coenzyme A from the host red cell cytosol is therefore not applicable in the case of *P. falciparum*-infected human erythrocytes.

**EXPERIMENTAL PROCEDURES**

Cell Culture and Preparation—Human erythrocytes (type O) infected with either the ITO4 (14) or FAF6 (15) lines of *P. falciparum* were cultured under 1% O₂, 3% CO₂, 96% N₂ in RPMI 1640 culture medium (Life Technologies, Paisley, UK or JRH Biosciences (CSL), Melbourne, Australia) supplemented with HEPES (40 mM), glucose (10 mM), glutamine (2 mM), gentamicin sulfate (25 mg liter⁻¹), and either human serum (5.5% v/v, pooled from different blood donors) or Albumax (0.5% w/v). All experiments with parasitized erythrocytes were carried out using trophozoite-infected cells (approximately 35 h postinvasion), with the sufficient of 1 mL of infected erythrocyte culture medium.

In experiments comparing infected with uninfected erythrocytes, infected erythrocytes (from the same donor) were incubated in parallel with uninfected erythrocytes (from the same donor) were incubated in parallel with *P. falciparum*-infected avian erythrocytes under identical conditions, for at least 24 h before the experiment was carried out. To ensure that the infected and uninfected cell suspensions were strictly comparable, the infected cells were centrifuged on a Percoll layer (as above) and harvested (from the bottom of the centrifuge tube) before experimentation.

Cell counts were made using either an improved Neubauer counting chamber or a Coulter Multisizer. Parasitemia was measured from methanol-fixed Giemsa-stained smears.

Saponin Permeabilization of Parasitized Erythrocytes—In a number of experiments parasitized erythrocytes were permeabilized by treatment with saponin, a plant-derived glycoside, using a method from that described by Hsiao et al. (19) Saponin interacts with cholesterol in the erythrocyte membrane to form lytic pores, but it leaves the parasite plasma membrane (which lacks cholesterol) intact.

Saponin (0.05% w/v) was added to parasitized erythrocytes (hematocrit ~10%) suspended in RPMI supplemented with glucose (20 mM) and HEPES (25 mM) at 37 °C. The suspension was centrifuged immediately (2,060 × g, 10 min) and then resuspended in an appropriate volume of RPMI prewarmed to 37 °C.

Approximately 92% of the parasites within the permeabilized cells excluded trypan blue, consistent with their plasma membrane remaining intact.

**Uptake Measurements in Intact Erythrocytes—**Uptake of pantotheine into intact infected and uninfected erythrocytes was monitored using [14C]pantothenate (NEN Life Science Products). All uptake measurements were carried out using cells washed (three times) and resuspended in RPMI 1640 at 37 °C. Cell suspensions were aliquoted into microcentrifuge tubes, and uptake commenced with the addition to the cell suspension of [14C]pantothenate (at 0.1 μCi ml⁻¹), giving a final extracellular pantothenate concentration of 3 μM. The final cell count was, in most experiments, approximately 2 × 10⁸ cells ml⁻¹. At appropriate time intervals aliquots (typically 0.15 ml) of the suspension were transferred to microcentrifuge tubes which, with the exception of those experiments in which the degree of phosphorylation of intracellular solute was determined (see next section), contained ice-cold RPMI (typical 0.45 ml) supplemented with 0.2 mM furosemide and layered over 0.2 ml of dibutyl phthalate. The tubes were capped (10,000 × g, 50 s) to sediment the cells below the oil, thereby terminating the uptake of radiolabeled solute.

After sedimentation of the cells below the oil, the aqueous supernatant solution was aspirated, and the radioactivity remaining on the walls of the tube was removed by rinsing the tubes four times with wash buffer. The dibutyl phthalate was aspirated, and then the cell pellet was lysed with 0.1% (v/v) Triton X-100 (0.5 ml) and deproteinized by the addition of 5% w/v trichloroacetic acid (0.5 ml) followed by centrifugation (10,000 × g, 10 min). Radioactivity was measured using a scintillation counter.

In one series of experiments the transport of [14C]pantothenate into intact parasitized erythrocytes was compared with that of [14C]choline. Uptake of [14C]choline into parasitized erythrocytes at 37 °C was measured using a method similar to that described previously (20). Cells were suspended in RPMI 1640, and the choline influx rate was estimated from the amount of [14C]choline taken up into infected cells during a 10-min incubation period. In all such experiments [14C]choline was added together with unlabeled choline to give an extracellular concentration of 1 mM. The contribution to net uptake of endogenous choline transporter and thereby minimize the contribution of this pathway to the influx of radiolabeled choline into the parasitized cells. Choline influx experiments were paired with pantothenate uptake experiments in which the rate of influx of pantothenate was estimated from the amount of [14C]pantothenate taken up during a 20-s incubation by cells suspended in RPMI 1640 supplemented with 1 mM choline.

In all experiments the amount of radiolabel trapped in the extracellular space within the cell pellets was estimated using either [14C]chlorinol or [14C]sucrose (at 0.25 μCi ml⁻¹ and 0.5 μCi ml⁻¹, respectively) as an extracellular space marker.

**Monitoring the Phosphorylation of Pantothenic Acid—**Phosphorylation of pantothenate by intact erythrocytes, saponin-permeabilized cells, and cell lysates was estimated using a combination of ZnSO₄ and Ba(OH)₂ (Somogyi reagent) which acts to precipitate phosphorylated compounds from solution (21, 22). In preliminary experiments it was shown that treatment of a solution of [14C]pantothenate with Somogyi reagent had no effect on the concentration of radiolabel in the solution, whereas treatment of a solution of the phosphorylated compound [35S]ATP-S resulted in a 99.5% depletion of radiolabel from the solution (20). Therefore, the test solution was prepared by adding ZnSO₄ and Ba(OH)₂ to the sample treated with Somogyi reagent and then incubating for 10 min at 37 °C.

Percichloric acid extracts of cells or lysates, preincubated with [14C]pantothenate (see below), were neutralized with a 5% w/v KOH solution. The neutralized extract was then divided into two aliquots, each typically 0.4 ml. To one was added 0.25 ml of 150 mM ZnSO₄ followed by 0.25 ml of 150 mM Ba(OH)₂. This sample (containing a heavy white precipitate) was allowed to stand for 5 min and then was centrifuged (10,000 × g, 15 min). The supernatant was transferred to a scintillation vial for β-counting. The second aliquot was made up to volume with 0.5 ml of H₂O and then transferred directly into a scintillation vial for β-counting.

The radioactivity in the Somogyi reagent-treated samples provided an estimate of the amount of (nonphosphorylated) [14C]pantothenate present. The difference between this amount and that present in the untreated samples provided an estimate of the amount of phosphorylated radiolabeled solute present.

**Phosphorylation of Pantothenate within Intact Parasitized Erythrocytes—**In experiments designed to monitor the phosphorylation of pantothenate taken up into intact cells it was necessary to ensure that all intracellular metabolism ceased at the time of sampling. In experiments with intact infected erythrocytes, uptake was halted by transferring aliquots of cell suspension to microcentrifuge tubes containing 0.5 ml of dibutyl phthalate layered over 0.5 ml of a 5.6% (w/v) solution of perchloric acid (PCA) made up in a saturated sucrose solution, then immediately centrifuging the tubes (10,000 × g, 50 s). The cells passed through the oil and into the PCA solution, thereby terminating the uptake, lysing the cells, and precipitating the cell protein. Then the tubes were washed (four times) as described above for the dibutyl phthalate. A 0.4-ml aliquot of the extract solution was transferred to a clean microcentrifuge tube where it was neutralized and then divided into two for quantitation of [14C]pantothenate phosphorylation using Somogyi reagent, as described above.

**Uptake and Phosphorylation of Pantothenate by Saponin-permeabilized Erythrocytes—**Initial attempts to adapt the method described...
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above (in which uptake and metabolism were halted instantaneously by centrifuging the cells through an oil layer into a dense PCA solution) for use with saponin-permeabilized erythrocytes were unsuccessful; the permeabilized cells have a lower density than intact cells and did not enter the PCA solution. In these experiments the metabolism of pantothenate was therefore halted by chilling the samples to 0 °C, at which temperature phosphorylation of pantothenate does not occur (see “Results”).

Saponin-permeabilized infected cells suspended in RPMI (at 37 °C) were combined with an equal volume of RPMI containing 0.2 μCi/ml [14C]pantothenate, giving a final activity of 0.1 μCi/ml and a final extracellular pantothenate concentration of 3 μM. At appropriate time intervals 0.3-ml aliquots of the permeabilized cell suspension were transferred into microcentrifuge tubes containing an oil layer (a 5:4 blend of dibutyl phthalate:dioctyl phthalate, having a density of 1.015 g/ml). The tubes were centrifuged immediately (15,800 × g, 10 min) and then chilled to 0 °C in an ice slurry. The supernatant was aspirated and the radioactivity remaining on the walls of the tube removed by rinsing the tubes five times with water. The oil was aspirated and the pellets were solubilized in 0.5 ml of 0.5% Triton X-100, then deproteinized by the addition of 5.6% w/v PCA (0.5 ml) followed by centrifugation (10,000 × g, 10 min). A 0.8 ml- aliquot of the supernatant was neutralized with 5% KOH then divided into two for quantitation of [14C]pantothenate phosphorylation using Somogyi reagent, as described above.

Phosphorylation of Pantothenate by Cell Lysates and Extracts—The presence of pantothenate kinase in the cytosol of normal uninfected erythrocytes and in the host cell and parasite compartments of trophozoite-infected cells was investigated using cell lysates and extracts prepared from: (i) high (>85%) parasitemia-infected cell suspensions; (ii) saponin-permeabilized parasitized erythrocytes; (iii) the erythrocyte cytosol fraction of saponin-permeabilized parasitized erythrocytes; (iv) a suspension of predominantly uninfected cells (3–5% parasitemia) harvested from infected cell suspensions (collected from beneath the Percoll layer used to generate suspensions having high parasitemias); (v) normal, uninfected erythrocytes, cultured in parallel with infected cell suspensions.

Phosphorylation of [14C]pantothenate by the various cell extracts was measured under conditions similar to those used by Bennett and Trager (5) in their original study of pantothenate metabolism by P. lophurae-infected duck erythrocytes. Cell lysates (from intact parasitized erythrocytes, saponin-permeabilized parasitized erythrocytes, uninfected erythrocytes) were prepared by diluting the cell suspension 1/10 in a 10 mM phosphate buffer, pH 7.4, followed by trituration (10 times) through a 25-gauge needle. The lysates were then centrifuged at 10,000 × g for 30 min, each time transferring the supernatant to another tube. The phosphorylation reaction commenced with the addition of a volume of the lysate supernatant (typically 250 μl) to a buffer containing 50 mM Tris, 5 mM ATP, 5 mM MgCl2, and 0.01 μCi/ml [14C]pantothenate (yielding a final pantothenate concentration of 0.2 μM). The solution had a pH of 7.4 and, unless specified otherwise, was prewarmed to 37 °C. At predetermined times, aliquots (200 μl) of the solution were transferred to a microcentrifuge tube containing 0.5 ml of 5.6% w/v PCA to halt the reaction. The tubes were centrifuged at 15,800 × g for 10 min and the resulting supernatant neutralized with 5% w/v KOH and processed as described above for the quantitation of [14C]pantothenate phosphorylation.

Rates of phosphorylation were estimated from the slope of the linear portion of the phosphorylation time courses.

Estimate of Intracellular Water Volumes and Intracellular Concentrations—In all experiments with intact infected and uninfected erythrocytes as well as saponin-permeabilized parasitized erythrocytes, the intracellular water content of the cell pellets was measured, thus enabling an estimate of the cell water volume and the calculation of intracellular solute concentrations. The total water volume of the pellet was estimated using H2O content and the extracellular water volume estimated using either [3H]inulin or [14C]sucrose. The △H2O, △[14C]inulin, and △[14C]sucrose were added to cells (at 0.5 μCl ml−1, 0.25 μCl ml−1, and 0.5 μCl ml−1, respectively) 10 min before sampling. The intracellular water volume was taken to be the difference between the total and extracellular water volumes.

The extracellular water volumes of uninfected erythrocytes, trophozoite-infected erythrocytes, and saponin-permeabilized parasitized erythrocytes were estimated as 75 ± 4 fl (n = 16), 75 ± 4 fl (n = 22), and 28 ± 3 fl (n = 12), respectively.

In all experiments with suspensions of intact malaria-infected erythrocytes the concentration of [14C]pantothenate within the parasitized cells (as distinct from those in the combined infected and uninfected cells in the cell pellet) was calculated by subtracting the contribution of the uninfected cells to the pellet water volume, based on the parasitemia and on the measured intracellular water volumes of infected and uninfected cells.

RESULTS

Uptake and Phosphorylation of Pantothenic Acid by Intact Parasitized Erythrocytes—Radiolabeled pantothenate was taken up rapidly into human erythrocytes infected with the mature (trophozoite) form of the malaria parasite (Fig. 2, open circles). The uptake time course was biphasic. There was an initial rapid influx (rate = 7.6 ± 1.5 μmol/(1012 cells · h); n = 3) lasting 1–2 min, during which the intracellular concentration of radiolabel rose to 0.5–0.6 that in the external medium. This was followed by a second, slower phase (rate = 1.4 ± 0.3 μmol/(1012 cells · h); n = 3), during which the intracellular concentration of radiolabel increased (linearly with time) to levels well in excess of the extracellular concentration.

In contrast with the parasitized cells, there was negligible uptake of the vitamin into normal, uninfected erythrocytes over a 20-min period (Fig. 2, closed circles).

The origin of the two components of uptake of pantothenate into parasitized cells was investigated using Somogyi reagent, a combination of ZnSO4 and Ba(OH)2 which, when added to cell extracts, has the effect of precipitating (and thereby removing from solution) any phosphorylated compounds present. The use of this reagent allows an analysis of the relative amounts of phosphorylated and nonphosphorylated radiolabeled solute in each sample (22). As shown in Fig. 3, the Somogyi reagent had little effect on the amount of radiolabel present in samples prepared from the initial phase of the uptake time course. However, in samples treated with Somogyi reagent the second phase of the uptake time course was abolished. The initial phase therefore corresponds to the transport of pantothenate into the infected cell. The second phase corresponds to the phosphorylation of the vitamin within the cell, presumably by pantothenate kinase, the first step in the conversion of the compound to coenzyme A (3, 4).

The transport of pantothenate into parasitized erythrocytes was via a pathway that is absent from normal, uninfected erythrocyte (Fig. 2). An obvious candidate for this pathway is the previously characterized NPP, induced by the intracellular parasite in the host cell membrane (9–13). The NPP have a very broad substrate specificity, accommodating a diverse variety of low molecular weight solutes. They are blocked by a range of anion transport inhibitors, one of the most effective of which is furosemide (12). Fig. 4 illustrates the effect of furo-
Semide on the time course for pantothenate uptake by malaria-infected cells. A 0.1 mM concentration of furosemide reduced the rate of transport of pantothenate into the cells by >95% (i.e. from 7.6 ± 1.5 μmol/10¹² cells · h) to 0.3 ± 0.1 μmol/10¹² cells · h; n = 3). Under these conditions the transport rate decreased to below the normal rate of phosphorylation of the compound (i.e. 1.4 ± 0.3 μmol/10¹² cells · h). Thus, in the presence of a transport inhibitor the flux of pantothenate into the cell became rate-limiting for the utilization of this essential vitamin by the parasitized cell.

The inset to Fig. 4 compares the dose-response curve for the inhibition by furosemide of the transport of pantothenate into parasitized cells with that for inhibition of the transport of choline, a monovalent cation that has been demonstrated previously to enter infected erythrocytes via the NPP (12, 20). The two curves were superimposable with IC₅₀ values (i.e. the concentration at which the inhibitory effect is half-maximal) of approximately 3 μM. The data are consistent with pantothenate and choline entering the parasitized cell via a common pathway.

When the uptake of [¹⁴C]pantothenate into infected cells was measured in the presence of an increased (10 mM) concentration of (unlabeled) pantothenate the initial rate of uptake of radiolabeled substrate was unaffected (Fig. 5), indicating that the influx pathway did not saturate at this substrate concentration. By contrast, the second phase of the radiolabel uptake time course was abolished, consistent with saturation of pantothenate kinase by the unlabeled solute.

Uptake and Phosphorylation of Pantothenic Acid by Saponin-permeabilized Parasitized Erythrocytes—The data presented thus far provide clear evidence for the entry of pantothenate into malaria-infected erythrocytes via a furosemide-sensitive, nonsaturable pathway and its subsequent phosphorylation (a saturable process) within the parasitized cell. The question remains, however, of where in the cell the phosphorylation occurs.

In early work with duck erythrocytes infected with P. lophurae it was found that pantothenate kinase (5), as well as the other enzymes involved in the conversion of pantothenate to coenzyme A (6), were present in the cytosol of the duck erythrocyte but absent from the parasite, prompting the suggestion that conversion of pantothenate to coenzyme A takes place exclusively in the host cell cytosol.

Quite different results were obtained here with P. falciparum. As shown in Fig. 6, parasites within saponin-permeabilized infected erythrocytes, lacking the enzymes of the red cell cytosol, accumulated radiolabeled pantothenate (in a predominantly phosphorylated form) from the medium. The maximal rate of accumulation of pantothenate by the parasites in this preparation, measured over the initial 30 min of the uptake time course, was 1.3 ± 0.3 μmol/10¹² cells · h (n = 3). This value was not significantly different from the rate of accumulation of pantothenate by intact infected erythrocytes during the second phase of the biphasic uptake time course (i.e. 1.4 ± 0.3 μmol/10¹² cells · h); p = 0.74, unpaired t test).

Phosphorylation of Pantothenate by Cell Lysates and Extracts—The results obtained with saponin-permeabilized cells...
showed a low phosphorylation activity (0.38 µmol/(10¹² cells) (3–5% parasitemia) isolated from the infected cell cultures (SP-pRBC (parasite); n = 5); lysates of predominantly uninfected cells isolated from infected cell cultures (pRBC (<5%) n = 5); lysates of normal uninfected erythrocytes (RBC; n = 5); and the red cell cytosol fractions both of saponin-permeabilized parasitized erythrocytes (SP-pRBC (RBC); n = 2) and saponin-permeabilized normal uninfected erythrocytes (SP-RBC; n = 2). The concentration of pantothenate added to each sample was 0.2 µmol.

FIG. 6. Uptake and phosphorylation of [¹⁴C]pantothenate by saponin-permeabilized malaria-infected erythrocytes. The different time courses indicate intracellular levels of nonphosphorylated [¹⁴C]pantothenate (Δ), phosphorylated [¹⁴C]pantothenate derivatives (■), and total radiolabeled solute (i.e. phosphorylated + nonphosphorylated [¹⁴C]pantothenate) (○). The amounts of phosphorylated and nonphosphorylated compound were determined using Somogy reagent (as outlined under “Experimental Procedures”). The data are averaged from three separate experiments and are shown ± S.E. The extracellular pantothenate concentration was 3 µmol, and the intracellular water volume of the parasite within the saponin-permeabilized erythrocytes used in the three experiments was 28 ± 7 fl.

FIG. 7. Phosphorylation of [¹⁴C]pantothenate by cell lysates and extracts. The graph shows the mean rates of phosphorylation (± S.E.) by: lysates of intact parasitized erythrocytes corrected to 100% parasitemia (pRBC (100%); n = 5); extracts of saponin-permeabilized (SP) infected erythrocytes, lacking the enzymes of the host cell cytosol (SP-pRBC (parasite); n = 5); lysates of predominantly uninfected cells isolated from infected cell cultures (pRBC (<5%) n = 5); lysates of normal uninfected erythrocytes (RBC; n = 5); and the red cell cytosol fractions both of saponin-permeabilized parasitized erythrocytes (SP-pRBC (RBC); n = 2) and saponin-permeabilized normal uninfected erythrocytes (SP-RBC; n = 2). The concentration of pantothenate added to each sample was 0.2 µmol.

The rate of phosphorylation by lysates of intact, parasitized cell suspensions (85–96% parasitemia) ranged from 2.5 to 3.8 µmol/(10¹² cells · h), some 8–12-fold higher than that in lysates of normal uninfected erythrocytes (0.32 ± 0.07 µmol/(10¹² cells · h); n = 5). Lysates prepared from predominantly uninfected cells (3–5% parasitemia) isolated from the infected cell cultures showed a low phosphorylation activity (0.38 ± 0.06 µmol/(10¹² cells · h); n = 5), similar to that of lysates from normal uninfected cells. The mean phosphorylation activity of lysates of the infected cells in the parasitized cell suspension was calculated to be 3.5 ± 0.2 µmol/(10¹² cells · h); Fig. 7), 11 times higher than that in the uninfected cells (p = 0.0003; paired t test).

It should be noted that the rate of phosphorylation of pantothenate by the lysates of the parasitized cells was some 2.5 times higher than the rate of phosphorylation of pantothenate within either intact or saponin-permeabilized parasitized erythrocytes. This is despite the fact that the concentration of pantothenate added to the lysates (0.2 µmol) was 15-fold less than that in the medium in which the intact or saponin-permeabilized cells were suspended (3 µmol). The concentration of (nonphosphorylated) pantothenate within the parasitized erythrocyte is less than that in the external medium (Fig. 3), and the concentration within the parasite itself may well have been substantially less than that in the extracellular solution. However, it is unlikely to have been below the 0.2 µmol used in the lysate experiments, and the explanation for the discrepancy in the phosphorylation rates in the two types of experiment probably relates to the drastically different conditions in the lysate compared with the parasite cytosol. In particular, the cell lysates were supplemented with supraphysiological levels of ATP (5 mM) as well as having subphysiological concentrations of any endogenous regulatory (inhibitory) factors that might be present within the parasite.

Lysates prepared from saponin-permeabilized parasitized erythrocytes (lacking the enzymes of the host red cell cytosol) demonstrated approximately 85% of the phosphorylation activity of those from intact parasitized cells (Fig. 7). Extracts of the host cell cytosol compartment of parasitized erythrocytes (obtained by saponin-permeabilization of high parasitemia-infected cell suspensions) phosphorylated [¹⁴C]pantothenate at a rate slightly (though not significantly) lower than uninfected erythrocyte extracts prepared by saponin lysis (Fig. 7).

From these results it is clear that the majority of the pantothenate phosphorylation activity within the malaria-infected cells resides within the intracellular parasite. The host erythrocyte cytosol does have a pantothenate kinase activity, but it is more than 10-fold lower than that of the parasite.

The theoretical possibility that the relatively low rate of phosphorylation of [¹⁴C]pantothenate by uninfected erythrocyte lysates was caused not by the red cell having low levels of the relevant kinase but, instead, by the presence of inhibitory factors (e.g. unlabeled pantothenate) in the uninfected erythrocyte cytosol is ruled out by the finding that the rate of phosphorylation of [¹⁴C]pantothenate measured in a sample in which extracts of saponin-permeabilized parasitized erythrocytes and normal uninfected erythrocytes were combined was similar to (and not significantly different from) the sum of the activities measured in each of the extracts alone (not shown).

The phosphorylation of [¹⁴C]pantothenate by extracts of saponin-permeabilized parasitized erythrocytes was inhibited completely by the omission of exogenous ATP from the incubation mixture, by placing the extracts in a boiling water bath for 10 min before the incubation, or by carrying out the incubation at 0 °C (not shown). The latter findings are consistent with the phosphorylation activity being enzyme-mediated.
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**DISCUSSION**

Pantothenic acid is one of relatively few low molecular weight solutes for which the intraerythrocytic malaria parasite *P. falciparum* has an absolute extracellular requirement. Omission of pantothenic acid from the external medium results in cessation of parasite growth (2). The pathways involved in the transport and metabolism of this essential vitamin in parasitized cells therefore hold significant interest as chemotherapeutic targets.

**Transport of Pantothenate into the Malaria-infected Erythrocyte**—In this study it was shown that pantothenate is impermeant to normal uninfected erythrocytes but enters malaria-infected erythrocytes rapidly via a pathway showing the characteristics (fructose sensitivity, nonsaturability) of the NPP induced by the intracellular parasite in the host erythrocyte membrane. The NPP have been described previously in some detail (9–13). They have been shown to be permeable to a very wide range of low molecular weight solutes, including a number of nutrients required by the intracellular parasite. However, most of the latter compounds (e.g. glucose, nucleosides, and those amino acids either absent from or present at very low levels in the host cell hemoglobin) gain entry into the malaria-infected erythrocyte via endogenous host cell transporters; there are few, if any, essential nutrients for which there is clear evidence that their transport via the NPP plays a physiological role in the parasitized cell. In identifying the NPP as the sole route of entry of the essential vitamin, pantothenate, into the malaria-infected erythrocyte the present study provides evidence for a critical physiological role for the NPP. Furthermore, the finding that in the presence of fructose the transport of the vitamin into the infected cell becomes rate-limiting for pantothenic acid utilization (Fig. 4) focuses attention on the possibility that the NPP may offer a suitable target for chemotherapeutic attack (20).

**Metabolism of Pantothenate within the Parasitized Cell**—Once inside the infected erythrocyte, pantothenate is phosphorylated, the first step in its conversion to coenzyme A. Early work with *P. lophurae*-infected duck erythrocytes led to the proposal that pantothenate kinase (5) and the other enzymes involved in the conversion of pantothenate to coenzyme A (6) were located exclusively within the host erythrocyte compartment. However this is not the case in *P. falciparum*-infected human erythrocytes. Experiments with saponin-permeabilized parasitized erythrocytes demonstrated that the intracellular parasite has the ability to take up pantothenate from the extracellular solution (indicative of the presence of a pantothenate transport pathway at the parasite surface) and to phosphorylate it (indicative of the presence of a pantothenate kinase within the parasite).

The rate of accumulation of the phosphorylated form of pantothenate by parasites in saponin-permeabilized parasitized erythrocytes (1.3 ± 0.3 nmoles/10^{12} cells · h) was similar to that measured in intact parasitized erythrocytes (1.4 ± 0.3 nmoles/10^{12} cells · h). There is therefore sufficient activity within the parasite to account for most, if not all, of the phosphorylation activity observed within the intact infected erythrocyte.

Experiments with cell extracts (carried out under conditions similar to those used in the earlier study of parasitized avian erythrocytes) revealed that although the host erythrocyte cytosol did contain a pantothenate kinase activity it was more than 10-fold less than that of the intracellular parasite and therefore insufficient to account for the phosphorylation activity observed in intact parasitized erythrocytes.

Together, these results allow the conclusion that in *P. falciparum*-infected human erythrocytes most, if not all, of the phosphorylation of pantothenate, *en route* to coenzyme A, occurs within the intracellular parasite. The data do not permit the determination of whether the phosphorylation of pantothenate within the intact parasitized cell is mediated exclusively by the parasite (as might be the case if the NPP provide the pantothenate with a direct route to the parasite, without it passing through the erythrocyte cytosol (23)) or whether there is a minor contribution from the enzymes present in the host cell cytosol (as might be the case if, as has traditionally been assumed, solutes entering the cell via the NPP enter the red cell cytosol, from where they are taken up by the parasite (24)). Nevertheless, they do show conclusively that the model postulated for parasitized avian erythrocytes, in which the parasite is dependent upon the surrounding host cell cytosol for a supply of preformed coenzyme A is not applicable to *P. falciparum*-infected human erythrocytes.

**Conclusions**—Pantothenate is impermeant to normal human erythrocytes but is taken up rapidly into *P. falciparum*-infected erythrocytes via NPP induced by the parasite in the host cell membrane. Once inside the parasitized cell the pantothenate is phosphorylated, the first step in its conversion to coenzyme A. Under normal conditions the rate of transport of pantothenate into the infected cell is much faster than its subsequent phosphorylation. However, in the presence of an inhibitor of the NPP the initial transport step becomes rate-limiting for pantothenate utilization. Although the red cell cytosol does show low levels of pantothenate kinase activity, the majority of the phosphorylation occurs within the intracellular parasite. The enzymes involved are yet to be identified at a molecular level but these, together with the pantothenate transport pathways in both the parasite and host cell membranes, hold significant promise as targets for chemotherapeutic attack.

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