Transport Pathways in the Malaria - Infected Erythrocyte: Characterization and their Use as Potential Targets for Chemotherapy

Hagai Ginsburg

Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

The intraerythrocytic malarial parasite is involved in an extremely intensive anabolic activity while it resides in its metabolically quiescent host cell. The necessary fast uptake of nutrients and the discharge of waste product, are guaranteed by parasite-induced alterations of the constitutive transporters of the host cell and the production of new parallel pathways. The membrane of the host cell thus becomes permeable to phospholipids, purine bases and nucleosides, small non-electrolytes, anions and cations. When the new pathways are quantitatively unimportant, classical inhibitors of native transporters can be used to inhibit parasite growth. Several compounds were found to effectively inhibit the new pathways and consequently, parasite growth. The pathways have also been used to introduce cytotoxic agents. The parasitophorous membrane consists of channels which are highly permeable to small solutes and display no ion selectivity.

Transport of some cations and anions across the parasite membrane is rapid and insensitive to classical inhibitors, and in some cases it is mediated by specific antiporters which respond to their respective inhibitors. Macromolecules have been shown to reach the parasitophorous space through a duct contiguous with the host cell membrane, and subsequently to be endocytosed at the parasite membrane. The simultaneous presence of the parasitophorous membrane channels and the duct, however, is incompatible with experimental evidences. No specific inhibitors were found as yet that would efficiently inhibit transport through the channels or the duct.

Keywords: malaria - erythrocyte - chemotherapy

During the last decade, malaria research has been intensified both at the applied and at the basic biological level. Although most of the resources have been devoted to vaccine development, some novel chemotherapeutic approaches have been entertained, based on a better understanding of the parasite biology and its interaction with the host. Since the pathogenicity of malaria is provoked mostly by the synchronous asexual development of the parasites inside the erythrocytes of the vertebrate host (schizogony), this stage of parasite development certainly deserves to be examined with a high priority in order to provide new rationales for therapeutic intervention. In this brief essay I shall review recent developments in the understanding of transport in and out the malaria-infected cell, and wherever possible, indicate the feasibility of using this knowledge for the design of new drugs.

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SEQUENTIAL AND PARALLEL PATHWAYS FOR THE MOVEMENT OF SOLUTES INTO AND OUT OF THE MALARIA-INFECTED RED BLOOD CELL

During the schizogony of *Plasmodium falciparum*, the most lethal human parasite species and hence responsible for most cases of mortality, a single intraerythrocytic parasite would produce asexually 16-32 progenies within 48 hours. Although this is a relatively slow rate of multiplication compared to other unicellular organisms, it nevertheless requires a considerable traffic of solutes in and out of the parasite and its host cell. This movement happens in a host cell whose innate metabolic activity is rather slight, although some metabolic pathways, e.g. glycolysis, the hexose monophosphate shunt, or some parts of purine salvage pathways, can be considerably accelerated given proper triggering. In some cases the native capacities of the host cell membrane probably suffice to meet the parasite's needs, e.g., glucose transport (in human but not in murine red cells), and accelerated uptake

is due to a larger metabolic sink. However, the permeability of the host cell membrane for other solutes, either substrates or waste products of host and/or parasite, may not be adequate for the malaria-infected cell. Such is the case, probably, for myo-inositol and for choline needed for phospholipid synthesis, for purine bases required for the synthesis of nucleic acids, and for some amino acids such as isoleucine which is relatively scarce in hemoglobin (digested by the parasite), or others which must be supplied from the outside to maintain parasite growth, at least in culture conditions (Divo et al. 1985). The high rate of lactate production in infected red blood cells IRBC (Pfaller et al. 1982) surpasses the capacity of the native carboxylate and anion transport systems, and ways must be secured to prevent extensive acidification of both parasite and host cell. Notably, murine RBC are only poorly permeable to glucose whereas their permeability to lactate is significantly higher compared to human RBC. Yet, mice and rats serve as hosts for various malaria species, suggesting that a universal strategy may be employed by the parasite to secure its physiological access to the extracellular world.

The parasite also links with the extracellular environment through proteins that it synthesizes and which could account for the phenomena of cytoadherance, erythrophagocytosis and immune suppression, and through proteins claimed to be endocytosed.

Various stratagems have been evolved by the parasite in order to secure an adequate traffic of solutes:

(a) The transmembrane movement of phospholipids which is usually very limited in the membrane of normal RBC, is significantly increased in infected cells, thus permitting the extensive biosynthesis of parasite membranes.

(b) The permeability of the host cell membrane is vastly increased to almost all solutes so far investigated, including anions, cations, carbohydrates, amino acids and nucleosides, although to a different extent in each case.

(c) Non-selective and relatively large pores, resembling those found in the outer membrane of mitochondria, have been identified in the parasitophorous membrane.

(d) The parasitophorous space has been shown to be connected directly to the extracellular milieu by means of a relatively large duct, that allows even proteins, let alone smaller solutes, contact with the parasite membrane, and to be subsequently endocytosed into the parasite. The same pathway could be used for the export of similar solutes.

(e) The parasite cell membrane is highly permeable to calcium, lactate, glucose and purine bases and nucleosides, and is equipped with various specific carrier mediated transport systems. An active proton extruder has also been suggested, and a Na+-K+-ATPase must also be invoked in order to account for the ability of the parasite to control a high K+:Na+ ratio in its cytosol.

(f) Extensive and diverse mechanisms have been identified to export various proteins from the parasite through the two concentric membranes that surround it and the host cell cytosol, to be either associated with the host cell membrane or transported through it.

(g) Finally, the parasite demonstrably endocytoses the cytosol of its host cell into its acidic food vacuole, where the cytosol is digested. In this way the parasite secures the provision of most amino acids and iron, although the contribution of other host cell solutes has not been investigated. The digestion of host cell hemoglobin also results in the production of reactive oxidative species, e.g. superoxide, hydrogen peroxide and OH radicals (Atamana & Ginsburg 1993). A partial protection against these oxidants is provided by ingested host cell superoxide dismutase, which is claimed to be partially resistant to proteolysis. However, it seems that this oxidative stress may be instrumental for the biochemical and structural modifications that the parasite must induce in the host cell for its own physiological advantage (Ginsburg 1990).

In what follows, these various pathways (excluding mechanisms (f) and (g) (which have been extensively reviewed recently: (Howard 1988), (Wiser 1991), (Elmendorf & Haldar 1993), (Knapp et al. 1991) will be briefly discussed in concert with their possible use as targets for chemotherapy.

TRANSPORT OF PHOSPHOLIPIDS

Malaria-infected erythrocytes take-up various phospholipids (PL) from the extracellular space. Uptake is mediated by PL-exchange protein (Moll et al. 1988) or HDL (Grellier et al. 1991). In normal RBC the internalization of PC (flip) is naturally very slow, while that of amino PL is rapid, ATP- and protein-dependent. It has been recently shown that the externalization (flop) of all PL is similarly energy- and protein-dependent (Connor et al. 1993). The

flip rate of PC is markedly and specifically accelerated in IRBC (Van der Schaft et al. 1987), (Simoes et al. 1991). Specificity, energy-dependence and susceptibility to chemical modifiers, suggest the induction of a specific flippase (Haldar et al. 1989). But an additional mechanisms may be operating. Increased flip-flop has been observed in sickle and thalassemic RBC, and has been alluded to oxidative stress. The parasite is known to exert an oxidative stress on its host cell (Hunt & Stocker 1990), simulating the processes observed in the variant erythrocytes. Alternatively, the insertion of parasite proteins into the HCM may cause structural imperfections along transmembrane proteins-PL interfaces. Such faults would result in an increase of the dielectric constant of the lipid barrier, thereby letting more intense flip-flop (Deuticke et al. 1992). Although the parasite is able to synthesize its own PL from essential imported building blocks (Vial et al. 1990), the imported phospholipids eventually reach the parasite, and are used as such or after further metabolism for the production of parasite membranes (Moll et al. 1988).

It has been recently demonstrated that the biogenesis of the parasitophorous vacuolar membrane (PVM) relies on PL supplied by the invading parasite, the merozoite (Dluzewski et al. 1992). It is not inconceivable that during this process the PVM becomes attached to the host cell membrane (HCM), either through veritable fusion or by some stable juxtaposition. Such contact could explain the many reports on the influx of fluorescent labelled PL which incorporate into the HCM, the PVM, the parasite membrane (PM) and to various inclusions in the host cell cytosol. The fast uptake of PL is initiated by increased rates of flip-flop across the HCM and further monomeric diffusion (possibly mediated by PL-transfer proteins(s)), or by lateral diffusion along the HCM-associated extension of the PVM.

It is very unlikely that the import of PL could serve as a target for chemotherapy, since no specific inhibitors for flippases have been identified yet, and because alternative mechanisms for PL synthesis do exist in the parasite (Vial et al. 1990).

INCREASED PERMEABILITY OF THE HOST CELL MEMBRANE TO POLAR SOLUTES

The most informative way of studying transport across membranes is the use of radiotracers, since this permits the full elucidation of the various kinetic parameters and of the biophysical nature (carrier, pore, simple diffusion) of the system studied (Stein 1986). However, the structural complexity of alternative transport pathways in IRBC may pose formidable theoretical and experimental difficulties in using radiotracers: 1) If the HCM is much more permeable than the PM, the use of radiotracers may be worthless for the study of the HCM. 2) An other complication may stem from the functional presence of the parasitophorous duct (see below). Labelled solutes could gain direct access into the parasite membrane, and time-dependent measurement of uptake may reflect solute transport both by the transcellular path and by the duct bypass. This difficulty could have been solved by measuring the volume of distribution of the transported solute as a function of time: A solute transported exclusively through the duct would distribute only into the parasite compartment, while one that translocates across the host cell membrane and then into the parasite, should distributes into the whole infected cell. This aspect is often overlooked or impractical due to metabolization (usually phosphorylation) of the substrate.

In spite of these difficulties, for many solutes, notably non-electrolytes, zwitterions and anions, an alternative methodology is available. When RBC are suspended in an isosmotic solution of a permeant solute (the ammonium salt in the case of anions), they lyse because of solute entry and the ensuing osmotic swelling exceeding the lytic volume. The half-time ($t_{1/2}$) of cell rupture is inversely proportional to the permeability coefficient of the solute (Stein 1967). The unique advantage of the osmotic lysis technique is that it monitors exclusively the permeability of the host cell membrane, irrespective of whether a parasitophorous duct (see below) exists or not.

CHARACTERIZATION OF PARASITE-INDUCED PATHWAYS USING THE ISOSMOTIC LYSIS TECHNIQUE

Using the isosmotic lysis technique, we have shown that the host cell membrane is highly permeable to most amino acids and to relatively small carbohydrates such as pentitols, pentoses, hexitols, hexoses (including D-glucose), but not di- or tri-saccharides (Ginsburg et al. 1983, 1985). Such lack of solute specificity and a relatively low enthalpy of activation of transport (10-11 Kcal/mole), suggests that the parasite induces a non-specific leak pathway

in its host cell membrane, or that it inserts in this membrane an aqueous channel of a relatively large diameter. This explanation is consistent with the total refractoriness of the pathway to specific inhibitors of the native RBC hexose transport system, and, on the other hand, its blocking by non-specific inhibitors such as phloretin (Cabantchik et al. 1983) or analogues of cinnamic acid (Kanaani & Ginsburg 1992).

All the available data on the lysis induced by carbohydrates and amino acids were subjected to a rigorous biophysical analysis (Ginsburg & Stein 1987). The body of data did not fit the model of an aqueous pore, neither did they seem to fit a model of "non-Stokesian diffusion" (Lieb & Stein 1986). However, the relative permeability was found to be correlated with the number of hydrogen bonds that the solutes can potentially form with water molecules. Hence, increased permeability could result from structural defects initiated in the host cell membrane due to the insertion and/or translocation of polypeptides produced by the intracellular parasite. Reconstitution of proteoliposomes from non-matching PL and proteins yield leaky membranes (Van der Steen et al. 1982, Van Hooghevest et al. 1984), as could also happen if parasite proteins do not fit the lipid composition of the host cell membrane. Interestingly, a mismatch of protein and lipid can also result in an increased transbilayer movement of phospholipids (Van der Steen et al. 1982), a phenomenon also observed in the membrane of malaria-infected red cells (Beaumelle et al. 1988).

The hydrophobic interaction between parasite proteins and host membrane lipids is supposedly weak and the PL head-group region, having allow dielectric constant, grants the selectivity filter which determines the membrane selectivity by determining solute partioning in the membrane (Stein 1986). The solubility of solutes in apolar solvents decreases with polarity of the solute. The partitioning of the tested solutes in a solvent that mimics the polarity of the polar head-group region and the relative permeability of the same solutes both depend (inversely) to the same extent on the number of hydrogen bonds that the solute can form with water, is expected from the mechanism of permeation. This mode of permeabilization would be compatible with the relative low specificity of the new pathways (no discrimination between optical enantiomers; Ginsburg et al. 1983), with the lack of saturability (Ginsburg et al. 1985, 1986c) and the insensitivity to chemical modifiers of proteins (Breuer et al. 1987). However, the insertion of specific, parasite-derived transport agencies, has also been suggested (Cabantchik 1990, Elford & Pinches 1992).

The new permeability pattern observed in infected cells, offers interesting prospects for the chemotherapy of malaria: drugs or pro-drugs could be designed to fit the permselectivity properties of the host cell membrane. Thus, although having cytotoxic effect, such compounds would not be able to penetrate into the host's cells and would hit only infected erythrocytes. Such inhibitors could be directed against physiological processes in the host cell that ensure the integrity of the parasite's intraerythrocytic haven, thus reducing the probability of evolution of drug resistance.

ALTERATIONS OF CONSTITUTIVE TRANSPORT SYSTEMS IN THE HOST CELL MEMBRANE

Activation of constitutive transporters could result either from a Km effect (lower Km, i.e., increased affinity to the substrate) and/or a Vmax effect (recruitment of silent carriers, change in turnover rate). In either case one should not expect *a priori* the altered transporter to preserve its susceptibility to specific inhibitors. The transport of tryptophan (Ginsburg & Krugliak 1983), glutamine and glutamate (Elford et al. 1985), nucleosides (Yamada & Sherman 1981, Gati et al. 1987, Gero et al. 1988) and glucose (Izumo et al. 1989, Tanabe 1990) were found to be considerably accelerated in malaria-infected RBC in parallel with parasite maturation. Interestingly, no acceleration was found with *p*-aminobenzoic acid (*p*ABA) which serves as a substrate for *de novo* folate synthesis in the parasite (Zhang et al. 1992). However, most of the innate transport of *p*ABA in erythrocytes occurs by rapid simple diffusion (and is not expected to be much influenced by structural defects), is high enough to supply the needs of the parasite.

In all these experiments, performed with radiolabelled substrates, two components of substrate uptake have been observed, one saturable and one non-saturable, and it was concluded that the constitutive host cell transport systems have been activated and that a parallel permeability pathway has been induced by the parasite (but the parallel transport through the parasitophorous duct can not be excluded). A deeper insight into the data on 2-deoxy-glucose transport in *P. yoelii*-infected

mouse erythrocytes (Izumo et al. 1989) indicates that the rate of translocation of the free carrier in the latter cells is 18.8-fold faster than in uninfected RBC. According to the kinetic analysis of the simple carrier (Stein 1986), the translocation of the unloaded carrier is the rate-limiting step in most carrier-mediated transport systems, and its increase results in both Vmax and Km rise.

The transport of nucleosides is also amplified in malaria-infected cells (Gero et al. 1988). The constitutive RBC system mediates the uptake of both purine and pyrimidine bases and nucleosides. While the parasite relies on its own synthetic capability of pyrimidines, its depends on the host cell for the supply of purines (Gero & O'Sullivan 1990). One of the major roles of RBC is the detoxification of adenosine released by some tissues into the blood stream. This is done by rapid uptake of adenosine and its metabolization to hypoxanthine by the purine salvage pathway. The normal serum levels of adenosine (1 M) and hypoxanthine (25 M) reflect the steady-state of these speedy processes. In IRBC the rate of adenosine transport is further increased and only part of it can be reduced by specific inhibitors of the native system (Gati et al. 1987, Gero et al. 1988). Unfortunately, it has not been tested whether the increased uptake is saturable or not (a Km effect). In *P. falciparum*-infected human RBC an increase in the number of transporters has been demonstrated (a Vmax effect?), but not in *P. yoelii*-infected mouse RBC. Since the permeability to tubericidine, a cytotoxic purine nucleoside analog, is also increased in infected cells, it has been suggested to use it in combination with nitrobenzylthiopurine analogues to treat malaria (Gero et al. 1989). The rationale for this combination is that the analogues will inhibit the influx of tubericidine into non-infected RBC and other host cell, thereby protecting them from the toxic effect of the drug, while its entry into infected cells will proceed unhampered with consequential parasite death. Although this rationale works perfectly well *in vitro*, it is rather unlikely that it could be extended to therapeutic application in view of the role of RBC in the detoxification of adenosine.

Classical inhibitors of the native systems had no effect on the non-saturable component of uptake and a reduced effectivity in blocking the saturable component. Other compounds of very different chemical nature, such as the bioflavonoids phloretin and phlorizin (Sylphen et al. 1988) or cinnamic acid and pyridoxalhydrazone derivatives (Kanaani & Ginsburg 1992) affect mostly the non-saturable component. Phloretin also inhibits the increased leakiness of red cells due to membrane barrier defects induced by oxidative stress, and it has been related to its ability to perturb and dehydrate the head group region of the membrane phospholipids (Deuticke et al. 1992). Hence, inhibition of the new pathways by this compound indirectly confirms that membrane structural defects underlie the permeabilization of the host cell membrane.

Malaria-infected cells are also more permeable to potassium probed by ^{86}Rb; (Bookchin et al. 1980, Kirk et al. 1991). This increased leak is not due to Ca^{2+}-dependent activation of the K^+ channel (Kirk et al. 1992) although the RBC Ca^{2+} content is substantially increased upon parasitization (Kramer & Ginsburg 1991). This increased leak in combination with the inhibition of the Na^+-K^+-ATPase (Dunn 1969), account for the large decrease in K^+ and the increase in Na^+ levels in the host cell compartment (Ginsburg et al. 1986a, Lee et al. 1988). It remains to be shown how the host cell retains its integrity faced with such disruption of the double-Donnan system (Dunham & Hoffman 1978).

The Ca^{2+} content of normal RBC is very low and probably insufficient for parasite growth. Upon invasion of the RBC by the merozoite, there is a transient rise in Ca^{2+} influx, but the excess calcium is cleared within the next 1-2 hours (Wasserman 1990). As the parasite matures, infected cells become more permeable to Ca^{2+} and their Ca^{2+} increases (Tanabe et al. 1982, Kramer & Ginsburg 1991). Here again, the native transport of RBC is saturable, while that of the infected cell is not (Kramer & Ginsburg 1991). No inhibitors have been identified so far that could specifically inhibit either the K^+, Na^+ or the Ca^{2+} pathways induced by the parasite.

Choline is an indispensable substrate for parasite PL synthesis (Vial & Ancelin 1992). Normal RBC have a constitutive choline carrier, and infected cells display enhanced choline uptake. According to one report on *P. knowlesi*-infected simian erythrocytes, it is saturable, displaying similar Km and 10-fold larger Vmax compared to uninfected RBC (possibly due to accelerated translocation of the unloaded carrier), and susceptibility to specific inhibitors (Ancelin et al. 1991). In *P. falciparum*-infected human RBC, no saturation was observed and sensitivity to specific inhibitors has not been tested

(Kirk et al. 1991). The choline analogues decyltrimethylammonium, decamethonium and hemacholinium 3, which are effective inhibitors of choline transport, also efficiently inhibited the growth of *P. falciparum* in culture and the incorporation of choline into *P. knowlesi* phosphatidylcholine (Ancelin et al. 1985), suggesting that choline transport could serve as a target for novel antimalarials, but since these inhibitors adversely affect cholinergic synapses, other less detrimental analogues should be found. Overall, it seems from the above deliberations, that when the permeability of the infected cell is only slightly increased to a certain solute, it may be the result of alteration of the constitutive transporter which is still amenable to inhibition by its specific inhibitors.

THE MODULATION OF ANION TRANSPORT IN IRBC

The capacity of RBC to transport anions is inherently very high (Knauf 1979). Nevertheless, the presence of a malaria parasite within these cells alters anion transport considerably (Kutner et al. 1983a,b, 1985). Monitoring continuously the efflux of the fluorescent anion analog NBD-taurine from infected cells, we have demonstrated (Kutner et al. 1985) that the rate of efflux increases with parasite maturation and that in parallel, the susceptibility of transport to DNDS (4,4'-dinitro-2,2'-stilbene disulfonic acid) decreases to nil in cells harboring mature parasite stages of parasite development. It could not be ascertained whether the native system is modified or if new permeability pathways are induced by the parasite in the host cell membrane. The number of native transport agencies is not altered due to parasite activity, and the integrity of band 3, the RBC membrane polypeptide mediating anion transport, is preserved throughout parasite development (Kutner et al. 1983a). The pitfalls in using tracers has been observed in a later series of experiments (Breuer et al. 1987), where influx was found to be several orders of magnitude faster than efflux. Logically, the two experimental protocols were monitoring different rate-limiting events, efflux revealing the transport across the parasite membrane while influx reflecting the permeability of the host cell membrane, both being insensitive to classical inhibitors of anion transport. Nevertheless, for yet unknown reasons, these compounds inhibited parasite growth and propagation with high correlation to their potency as inhibitors of the native anion transport of RBCs (Cabantchik et al. 1983).

The possibility that inhibition was exerted on lactate efflux (lactate being the major end product of parasite and host cell glucose oxidation) can be refuted. Lactate transport was shown to be 600-fold higher in infected cells despite the presence of the native transporter inhibitor DNDS and only partially sensitive to specific inhibitors of the native lactate transporter (Kanaani & Ginsburg 1991,1992; Poole & Halestrap 1993). Although the various derivatives of cinnamic acid were found to effectively inhibit parasite growth in culture (Kanaani & Ginsburg 1992), it is rather unlikely that they could serve as antimalarials because of their effect on the lactate transport across the plasma and the mitochondrial membranes of various host cells.

POSSIBLE PHARMACOLOGICAL IMPLICATIONS OF THE PARASITE-INDUCED TRANSPORT PATHWAYS

Undeniably, the permselectivity properties of the membrane of IRBC is distinctly different from those on uninfected RBC or any other type of mammalian cell. Only in the cases of nucleoside and choline transport, these alterations have been shown to provide targets for new antimalarial chemotherapy, though even in these cases, *in vivo* tests are far from being conclusive. However, the unique properties of the new pathways could provide for the targeting of tailor-made pharmacological agents specifically into IRBC. Such agents could be aimed against targets either in the host cell or in the parasite, the former being preferable because they would not provide for the evolution of drug resistance through selection. A straightforward approach would be to glycosylate a toxic agent thus rendering it less permeable into host cells (Matsumoto et al. 1991). The glycosylated compounds should be able to penetrate through the new permeability pathways, and act either directly, or after hydrolysis by red cell and/or parasite glucosidase(s).

This tactic for drug targeting has been experimentally demonstrated. Thus, phloridzin and its analogues penetrate into IRBC through the new pathways and inhibit parasite growth both by blocking these same pathways and by affecting parasite targets (Kutner et al. 1987). The zinc chelator dipicolininc acid does not enter into uninfected RBC, but readily permeates into IRBC and inhibits

parasite growth (Ginsburg et al. 1986b). Sometimes no direct effect of a drug can be observed on the parasite, yet the treated infected cell is eliminated: The pyridine glucosides of fava beans, vicine and convicine, penetrate into infected RBC where they are hydrolyzed by host cell and parasite ß-glucosidase to yield the highly oxidative aglycones divicine and isouramil. Whereas parasite growth is not inhibited by the glucosides, probably because the antioxidant defence capacity of the parasite suffice to counteract the deleterious effect of the aglycones, the phagocytosis of the IRBC is considerably enhanced, plausibly by the oxidative alteration of the host cell membrane (Ginsburg et al. unpublished observations). These three examples clearly demonstrate the feasibility of targeting of drugs or pro-drugs, and could be extended by a tactful choice of more specific and/or active and potentially chemotherapeutic agents.

POSSIBLE REASONS FOR TRANSPORT-RELATED ALTERATIONS OF THE HOST CELL MEMBRANE

Alterations of the native erythrocyte membrane transporters and/or the induction of new (leak?) pathways could stem from various processes occurring in the parasitized cell: (a) Oxidative stress demonstrably alters the permselectivity of RBC (Deuticke et al. 1983) and increase the flip-flop of PL. Oxidative stress is known to be exerted by the parasite on its host cell (Hunt & Stocker 1990) and increased flip-flop has been observed in the membrane of parasitized red cells (Beaumelle et al. 1987). (b) The host cell membrane is depleted of cholesterol (Holz 1977) with consequent fluidization (Howard & Sawyer 1980, Allred et al. 1983, Sherman & Greenan 1984). These alterations could modify either the basal permeability through an overall fluidization, or affect the carrier-mediated processes which may require cholesterol specifically or that are influenced by membrane fluidity (Yeagle 1985). (c) The parasite produces and exports polypeptides into and through the host cell membrane (see Howard 1988, Wiser 1991, Knapp et al. 1991, Elmendorf & Haldar 1993, for reviews). These could alter the kinetic properties of native transporters, or introduce structural defects into the host membrane. (d) Conceivably, some of the polypeptides inserted by the parasite could act as specific transport systems. Isolation of the host cell membrane, its fractionation into lipid and protein components, and reconstitution of the various components, could resolve these different alternatives.

THE PARASITOPHOROUS DUCT

Exposure of IRBC to fluorescently-labelled phospholipids results in a marked pattern of labelling, involving vesicular and tubular structures in the host cell cytosol and the parasite membranes. A direct tubular connection between host cell surface and the parasitophorous space has been recently thus demonstrated (Pouvelle et al. 1991) which admits also proteinaceous macromolecules. Fluoresceinated dextrans, rhodaminated protein A and fluorescently-labelled latex beads (30 nm) were shown by confocal microscopy to fill a continuous tubular structure bridging the host cell membrane and the parasitophrous space. Rhodamine-dextran was eventually endocytosed by the parasite in an energy-dependent process, e.g., requiring physiological temperature and adequate ATP levels. Pre-accumulated label was similarly released from infected cells, implying an exocytotic process. These results suggest a direct contact of the parasite membrane to the extracellular milieu by means of a parasitophorous duct. However, macromolecular uptake could not be demonstrated by similar techniques in other investigations (Grellier et al. 1991; Haldar et al. 1989), and it has been recently contended that the manifestation of the duct may be an experimental artifact (Fujioka & Aikawa 1993). Obviously, additional work is warranted to establish the physiological role of the duct.

PERMEABILITY OF THE PARASITOPHOROUS MEMBRANE

The parasitophorous membrane is the next barrier that must be hurdled by solutes that ingress across the host cell membrane. Since it is tightly juxtaposed to the parasite cell membrane, it is hard (and presently impossible) to isolate and investigating its permeability properties tenders a formidable task. Nevertheless, using the cell attached patch clamp technique, it has been shown recently to hold relatively wide channels (Desai et al. 1993). The channel has a high conductivity (140 pS), it admits equally well cations and anions, including lysine and glucuronate, is present at high density, and is open 98 % of the time. It seems to represent the major transport thoroughfare between the host cell cytosol and the parasite membrane, and reminisces the porin channels of Gram-negative and

mitochondria outer membranes. With these channels, the PVM can be viewed as a sieve that prevents the loss of enzymes and metabolites which are indispensable for host cell function, yet securing the translocation of solutes that must enter the parasite or exit from it.

While the presence of the PVM channels is physiologically sensible, it can not coexist with the parasitophorous duct, at least not by experimental criteria. For example, the PVM channels are permeable to Ca^{2+}, and hence, the host cell cytosol Ca^{2+} concentration should equilibrate with that of the extracellular medium. Yet, the host cell cytosol free Ca^{2+} in *P. falciparum*-infected RBC is 1-2 M (Kramer & Ginsburg 1991) or less (Adovelande et al. 1993), while that of the culture medium is 400 M. The PVM channel is also permeable to Na^+ and K^+, and the levels of these cations in the host cell cytosol should be equal to that of the bathing medium if the duct exists. This is indeed observed at the most advanced stages of parasite development (Lee et al. 1988) but not at the trophozoite stage (Ginsburg et al. 1986b) when the duct is perceived. Decrease in K^+ and increase in Na^+ could be fully explained by the increase in the leakiness of the host cell membrane to these cations and the inhibition of the sodium pump. Although the permeability of the PVM channel to small phosphorylated solutes has not been tested (the channel is highly permeable to tris whose molecular radius is of the size of phosphate), its high conductivity and lack of charge discrimination would suggest that intermediates of host cell metabolic processes (glycolysis, hexose monophosphate shunt, HMS) could also translocate through the channel and exit the infected cell through the duct. In fact, direct measurements show that the PVM is permeable to ATP and ADP (see below) and indirect evidence suggest that it also permeable to adenosine and inosine monophosphate (Ginsburg et al. unpublished observations). If this were true, one would expect total depletion of the host cell compartment from glutathione (its synthesis should be blocked) and massive oxidation of hemoglobin to methemoglobin (due to lack of NADH required as a cofactor for methemoglobin reductase). Both expectation are not met by direct measurements, and the HMS activity of the host cell compartment is actually increased several folds (Atamna & Ginsburg, unpublished observations).

PERMEABILITY OF THE PARASITE MEMBRANE

The knowledge of the transport characteristics of the parasite membrane (actually the PVM-PM couple) is very limited, most of it has been obtained by permeabilizing the membrane of the host cell to solutes of up to 1600 molecular weight by treating the cells with Sendai virus (Kanaani & Ginsburg 1989), thus allowing direct access of extracellular solutes to the parasite membrane. Using Sendai virus-treated infected RBC it has been demonstrated that the parasite membrane contains and ADP/ATP antiporter displaying the characteristics of the classical antiporter of mitochondria (Kanaani & Ginsburg 1989, Choi & Mikkelsen 1990), and suggested that by means of this system the parasite supplies ATP to the host cell whose glycolysis is impaired due to acidification and reduction of K^+ levels. Similarly, a Na^+/H^+ antiporter has been demonstrated and assigned a role in pH homeostasis of the parasite (Bosia et al. 1993) using the Na^+-gradient across the parasite membrane (probably maintained by a Na^+-K^+-ATPase) for the extrusion of protons. Using an indirect approach of measuring the distribution of 2-deoxyglucose (Izumo et al. 1988) and calcium (Tanabe et al. 1982) as affected by protonophores, it has been suggested that the parasite membrane also includes a glucose/H^+ symporter and a Ca^{2+}/H^+ antiporter, regulating glucose uptake and Ca^{2+} egress, respectively. Since a proton motive force is required for these processes to be of any physiological significance, an active H^+-pump has been assigned to the parasite membrane, but its function has been questioned (Bosia et al. 1993) and its presence has not been demonstrated directly. In fact, the Na^+/H^+ could suffice to generate the necessary pH gradient for glucose uptake and Ca^{2+} extrusion.

Using the same preparation, the transport of lactate (Kanaani & Ginsburg 1991) and Ca^{2+} (Kramer & Ginsburg 1991) across the parasite membrane has been investigated. The transport of both solutes was found to be non-saturable and while the rate of lactate translocation was similar to that of the infected cell membrane, that of Ca^{2+} was substantially more rapid. At the present time no specific inhibitors of solute transport across the parasite membrane have been found (except those inhibiting the antiporters), and the nature of these transport systems is virtually unknown. Further in-

vestigations are required to clarify the PM transporters in the hope that specific inhibitors may be found and eventually tested for their antimalarial potential.

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