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Salvage syntheses and their relationship to nucleic acid metabolism

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The intraerythrocytic stages of plasmodia are capable of synthesizing purine nucleotides and apparently deoxycytidylate by salvage syntheses. Data obtained by studying the incorporation of radioactive precursor molecules into intact cells and kinetic experiments on purified enzyme preparations suggest biosynthetic routes which, generally, are similar to those of the host's cell metabolism. However, details on the regulation of both the uptake of nucleosides and bases into the intraerythrocytic stages of plasmodia and of the metabolic routes involved in this incorporation are still lacking.

Mammalian erythrocytes do not synthesize DNA and RNA; however, they are able to synthesize nucleotides. The synthesis of purine nucleotides is not performed *de novo*, but via metabolic salvage pathways. Mature erythrocytes, therefore, rely on an external source of preformed purines that originate from the liver and probably other organs (*1*, *2*). The passage of nucleosides and bases across the plasma membranes is a process mediated by transport elements of the plasma membrane and is apparently closely linked to reactions that transform nucleosides and bases into impermeable nucleotides (*3*, *4*).

The intraerythrocytic stages of plasmodia possess high rates of DNA-synthesis and protein synthesis, and they require a vast supply of precursor molecules. The host erythrocytes have the remarkable capacity of providing the essential precursor molecules in amounts that will allow the successful growth and multiplication of the parasite within the cell. While the synthesis of dihydrofolate is an example of a parasite-specific sequence of reactions, the transformation of energy and the synthesis of nucleotides in the malaria parasite occur via metabolic routes very similar to those of their host cells.

The experiments performed by Büngener & Nielsen (*5*, *6*) clearly indicated the existence of a salvage synthesis of purine nucleotides and the apparent lack of the incorporation of ^{3}H-thymidine into DNA of rodent plasmodia. Incorporation studies have proved to be of value in studying the nucleotide metabolism of avian and mammalial plasmodia and conditions for the cultivation of plasmodia, and have yielded information on the mode of action of antimalarial drugs (*7–18*). Pyrimidine nucleotides are obviously synthesized *de novo* (*7*, *10*, *19*).

The separation of nucleotides, nucleosides, and bases by TL-chromatography on PEI-cellulose-coated Polygram sheets greatly facilitated the analytical work essential in performing sensitive enzyme assays and detailed studies on the fate of incorporated radioactive precursor compounds (*20*, *21*). By combining data from incorporation experiments in *P. berghei* and kinetic data obtained from enzyme studies with the supernatant of plasmodial homogenate and partly purified enzymes of *P. chabaudi*, the following metabolic scheme for purine salvage synthesis in the malaria parasite can be suggested (Fig. 1) (*22–26*): hypoxanthine, obtained either directly or by a sequence of perhaps membrane-associated reactions in which adenosine is taken

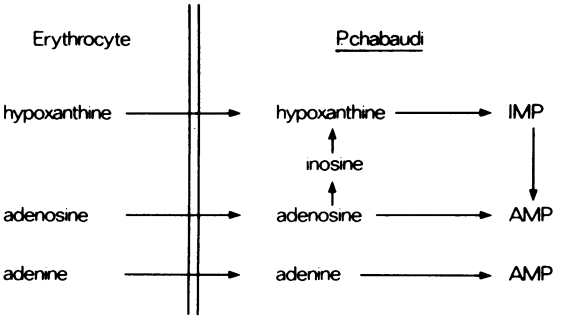

Fig. 1. Synthesis of adenosine 5'-monophosphate (AMP) in *Plasmodium chabaudi* (IMP = inosine 5'-monophosphate).

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up by the parasite, deaminated to inosine, and dephosphorylated to hypoxanthine, can be transformed to inosine 5'-monophosphate (IMP) by the catalytic action of hypoxanthine phosphoribosyltransferase (EC 2.4.2.8). IMP can be transformed to adenosine 5'-monophosphate (AMP) via the reactions that are catalysed by adenylosuccinate synthetase (EC 6.3.4.4) and adenylosuccinate lyase (EC 4.3.2.2). AMP can also be synthesized either from adenosine by a phosphorylating reaction catalysed by adenosine kinase (EC 2.7.1.20) or from adenine by adenine phosphoribosyltransferase (EC 2.4.2.7). Such salvage syntheses have also been reported from erythrocytes, leucocytes, and thrombocytes. A similar pathway of adenosine utilization is found in a system that regulates the coronary blood flow of the heart muscle (*27*). Guanosine 5'-monophosphate can be synthesised by the action of hypoxanthine phosphoribosyltransferase and via IMP. The latter reaction has not been confirmed yet in plasmodia.

The salvage syntheses of purine nucleotides provide the precursor molecules for the biosynthesis of nucleic acids and the nucleotide coenzymes. An important precursor molecule of the latter group is guanosine 5'-triphosphate, the initial compound of the plasmodia-specific synthesis of dihydrofolate.

Autoradiographic studies with ^{3}H-thymidine (*5*) and the failure to measure activities of thymidine kinase (EC 2.7.1.75) suggest the absence of a salvage synthesis of thymidylate in plasmodia. These observations furnish an additional explanation for the inhibitory effect on the DNA synthesis of plasmodia by analogues of *p*-aminobenzoate and folate.

Deoxycytidine has been reported to be the major deoxynucleoside in the circulating blood (*28, 29*). The uptake and very low rates of transformation of deoxycytidine in *P. chabaudi*-infected red cells *in vitro* have been observed (*30*). This pathway could provide deoxycytidylate, which becomes deaminated to deoxyuridylate, the substrate of the thymidylate synthetase reaction (*30*) (Fig. 2).

Any discussion on the regulation of the salvage synthesis of purine nucleotides will remain incomplete without considering the mechanisms of membrane transport and aspects of compartmentalization. However, kinetic data obtained from partly purified enzyme preparations suggest that the utilization of adenosine may proceed at low concentrations via the reaction catalysed by adenosine kinase and at higher concentrations via that catalysed by adenosine deaminase (EC 3.5.4.4) (Table 1). Adenosine kinase, which may operate in close association with the membrane, is capable of using adenosine analogues as substrates. However,

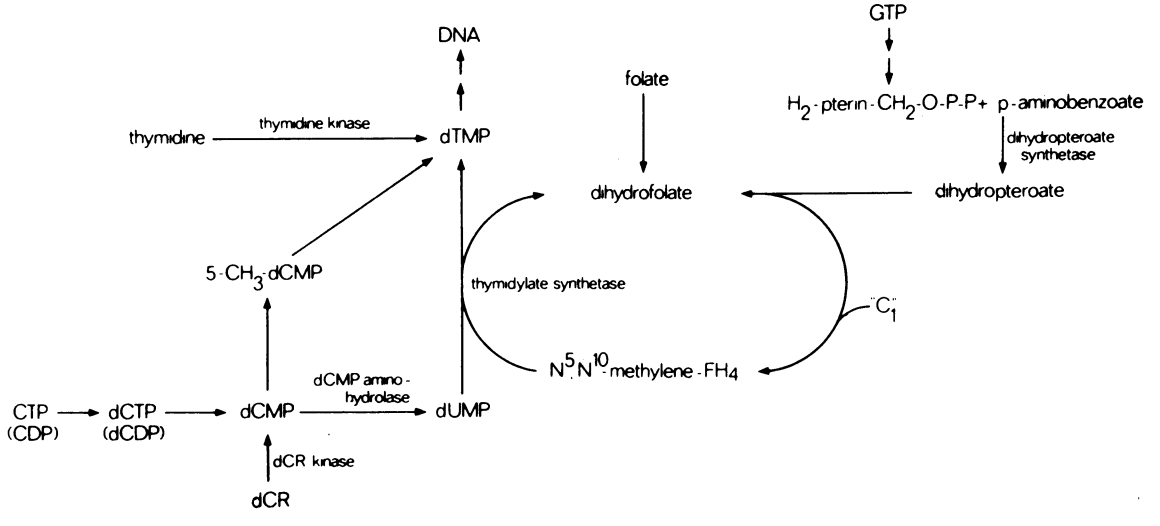

Fig. 2. Biosynthesis of deoxynucleotides (CDP = cytidine 5'-diphosphate; CTP = cytidine 5'-triphosphate; dCDP = deoxycytidine 5'-diphosphate; dCMP = deoxycytidine 5'-monophosphate; dCR = deoxycytidine; dCTP = deoxycytidine 5'-triphosphate; dTMP = thymidine 5'-monophosphate; dUMP = deoxyuridine 5'-monophosphate; GTP = guanosine 5'-triphosphate).

Table 1. Michaelis–Menten constants of adenosine kinase and adenosine deaminase from human erythrocytes (*31*) and *P. chabaudi*

	Human erythrocytes	*P. chabaudi*
adenosine kinase	1.9×10^{-6} mol/litre	6.5×10^{-6} mol/litre
adenosine deaminase	4.0×10^{-5} mol/litre	1.3×10^{-4} mol/litre

the further transformation of the nucleotide analogues may vary because of differences in the specificity of the adenylate kinase reaction (*3*). Cordycepin and tubercidin, both analogues of adenosine and substrates of adenosine kinase, have been assayed in laboratory experiments for their antimalarial and schistosomicidal effectiveness (*15*, *31*).

Plasmodia, like human erythrocytes, possess two distinct enzymes for a phosphoribosyltransferase reaction using hypoxanthine (guanine) and adenine as a source of purines (*25*). The Michaelis–Menten constant for hypoxanthine of the hypoxanthine phosphoribosyltransferase reaction was 2.5×10^{-6} mol/litre, for adenine of the adenine phosphoribosyltransferase reaction it was 5.0×10^{-6} mol/litre. Only the former reaction was competitively inhibited by the purine analogues 6-mercaptopurine, 2-amino-6-mercaptopurine, and 6-thioguanine (Table 2). Isoenzymes of hypoxanthine phosphoribosyltransferase that have been reported from various systems have not been found in *P. chabaudi*. The molecular weights of 71 000 for hypoxanthine phosphoribosyltransferase and 34 000 for adenine phosphoribosyltransferase correspond with those obtained from human erythrocytes (68 000 and 34 000, respectively (*25*, *32*, *33*)). The isoelectric point of plasmodial hypoxanthine phosphoribosyltransferase of 5.25 differed from those reported from human erythrocytes (*25*, *32*, *33*). The kinetic data provided suggest that hypoxanthine phosphoribosyltransferase functions as a key enzyme in the reutilization of purines. Allopurinol added to the drinking-water of mice stimulated the parasitization of mature erythrocytes in the *P. berghei*-infected mouse and the multiplication of *P. vinckei* and *P. chabaudi* in mice and rats. This indicates also that purines from exoerythrocytic sources are limiting factors of the multiplication of intraerythrocytic plasmodia (*34*). The concentration of phosphoribosylpyrophosphate (PRPP) and of ribose 5′-phosphate may be of regulating importance (*35*). In Lesh–Nyhan red cells with decreased activity of hypoxanthine phosphoribosyltransferase, the concentration of PRPP is elevated by up to ten times. This elevation of PRPP may increase the activity of adenine phosphoribosyltransferase by stabilizing the enzyme (*36*).

Table 2. Michaelis–Menten constants and inhibitor constants of partly purified hypoxanthine phosphoribosyltransferase (HGPRTase) and adenine phorphoribosyltransferase (APRTase)

Enzyme	Substrate or inhibitor	K_m ($\times 10^{-6}$ mol/litre)	K_I ($\times 10^{-6}$ mol/litre)
HGPRTase	hypoxanthine	2.5	
	guanine	2.0	
	6-mercaptopurine		7.0
	2-amino-6-mercaptopurine		4.0
	6-thioguanine		5.0
APRTase	adenine	5.0	

Plasmodia, obviously, are capable of providing the nucleotides that they need for the synthesis of their nucleic acids, mainly by simulating reactions that also exist in their host cells. However, minor differences may exist in the sequence and regulation of some reactions—e.g., the synthesis of AMP from IMP has not been reported from human erythrocytes because of an apparent lack of adenylosuccinate synthetase and lyase—enzymes that have been observed in rabbit reticulocytes and in preparations of *P. chabaudi*.

RÉSUMÉ

LES SYNTHÈSES PAR RÉCUPÉRATION ET LEURS RELATIONS AVEC LE MÉTABOLISME DES ACIDES NUCLÉIQUES

Les stades intra-érythrocytaires des plasmodies sont capables de synthétiser par récupération les nucléotides puriques et apparemment le désoxycytidylate. Les données fournies par l'étude de l'incorporation de molécules précurseurs radio-actives dans des cellules intactes, et par les expériences de cinétique sur des

préparations enzymatiques purifiées, semblent indiquer qu'il existe des voies biosynthétiques qui, en général, sont similaires à celles du métabolisme de la cellule hôte. Toutefois, on manque encore de détails sur la régulation de l'incorporation des nucléosides et des bases dans les stades intra-érythrocytaires des plasmodies et sur celle des voies métaboliques impliquées.

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