SUMMARY

1. This study shows for the first time variations in the p-aminobenzoic acid (PABA) requirement of Plasmodium falciparum. The minimal requirement for growth of the sulfadoxine sensitive parasite was 25.0 ng/ml whereas the drug resistant parasite no longer utilized PABA as a precursor for the biosynthesis of dihydrofolate. The ability to uptake PABA analogs, e.g., sulfadoxine, was reduced to one fifth in the resistant parasite, suggesting a mechanism with which the parasite circumvented drug action.

In addition to this major finding, a new in vitro test and culture system has been developed to study the interactions between folate cofactors, antifolates and growth of P. falciparum. Results of the experiments are summarized in parts 2 to 8.

2. Thai strains of Plasmodium falciparum were rather uniformly resistant to pyrimethamine. The minimal inhibitory concentrations ranged from 10 to 60 nmole/ml. The sensitivity of the isolates to pyrimethamine was found to increase after three months of continuous culture in RPMI. That is, a concentration of pyrimethamine as low as 0.02-0.05 nmole/ml was enough to cause 50% inhibition of parasite population growth. Maintenance of pyrimethamine resistance could be achieved by culturing the isolates under drug pressure for three months. On withdrawal of the drug, the pyrimethamine resistance was stable. This observation, together with the finding that pyrimethamine

resistant parasites could be selected from the sensitive Gambian strain, lead to the suggestion that a mixed population of pyrimethamine sensitive and resistant falciparum existed in most patients. The resistant parasite lines could be selected from pre-existing mutants in a single isolate.

3. Cross resistance of pyrimethamine and amethopterin, a DHFR inhibitor not used for malarial chemotherapy was studied. The result gave evidence of cross resistance among drugs sharing the same mode of action.

4. In this study, the culture technique for P. falciparum was improved by the use of a p-aminobenzoic acid(PABA) free basic medium (Waymouth) in place of RPMI. Long term cultivation of P. falciparum in Waymouth medium was carried out. The results indicated that growth of asexual stages of both sensitive and resistant parasites in Waymouth was as good as that in RPMI when measured by the rate of glucose utilization, lactic acid production, and the extent of ^{3}H-methionine and ^{3}H-isoleucine incorporation. Thus, the PABA present in supplementing serum was sufficient for normal growth of both sensitive and resistant parasites.

It was demonstrated that Waymouth culture had other advantages over RPMI, e.g., daily change of the medium was not needed for the first 96 hrs of culture.

5. Waymouth medium was used in the assessment of pyrimethamine and sulfadoxine sensitivity in this study. Responses of the parasites to both drugs were demonstrated to increase significantly in Waymouth. This was shown to result because Waymouth medium did not contain PABA to compete with the drug uptake.

6. Study of the influence of PABA on drug activity indicate that PABA at a concentration higher than 40 ng/ml was able to interfere with the activities of sulfadoxine, pyrimethamine and a combination of the two. Extensive study on this aspect demonstrated that the effectiveness of sulfadoxine and pyrimethamine in a ratio of 20:1 was not affected by concentrations of PABA less than 40 ng/ml. Accordingly, previous reports on drug sensitivities both in vivo and in vitro by other authors, who disregarded PABA concentrations in their test systems must be reinterpreted.

7. The experiment on PABA requirements implied that resistant parasites did not depend on PABA whereas sensitive ones required at least 25 ng/ml for normal development. An alternate pathway for folate-cofactor biosynthesis in resistant P. falciparum was suggested.

The comparative study of ^{14}C-sulfadoxine incorporated into erythrocytes parasitized with sulfadoxine sensitive and sulfadoxine resistant P. falciparum gave some insight into the resistance mechanism. ^{14}C-sulfadoxine incorporated by erythrocytes harboring

resistant parasites was 5.5 times less than that for erythrocytes harboring sensitive parasites.

8. One series of experiments gave evidence that cross resistance may not occur between sulfadoxine and other sulfonamides. Also, the concentration of drug needed to obtain 50% inhibition was lower for sulfadoxine than for sulfadiazine and sulfisoxasole. It was thus recommended that sulfadoxine still be the drug of choice among the sulfonamides.

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