Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1960, **22**, 621-631

Mitigation of the Haemolytic Effect of Primaquine and Enhancement of its Action against Exoerythrocytic Forms of the Chesson Strain of *Plasmodium vivax* by Intermittent Regimens of Drug Administration*

A Preliminary Report

ALF S. ALVING, M.D.,[1] CHARLES F. JOHNSON, ALVIN R. TARLOV, M. D.[2]
GEORGE J. BREWER, M.D.,[3] ROBERT W. KELLERMEYER, M.D,[4] & PAUL E. CARSON, M.D.[5]

Primaquine—an 8-aminoquinoline derivative—is one of the most effective drugs for use against the tissue stages of the malaria parasite. Unfortunately certain persons suffer from an inherited defect of metabolism which renders them susceptible to haemolysis after ingestion of the 8-aminoquinolines, certain other drugs and some vegetables. Susceptibility appears to be inherited by a partially dominant sex-linked gene of variable expression. In persons with full expression of this defect, intravascular haemolysis may be of such severity as to mimic blackwater fever.

It has been shown that the haemolysis caused by daily doses of primaquine is self-limited, provided that such doses are not excessive, by virtue of the fact that the younger erythrocytes are relatively resistant to destruction by the drug.

Therapeutic studies reported in the present paper indicate that the toxicity is markedly diminished by regimens requiring administration in weekly doses (together with the standard suppressive dose of chloroquine or one of its congeners) while its therapeutic effectiveness in the radical cure of Chesson vivax malaria is increased.

A weekly dose of 45 mg primaquine proved highly effective against severe Chesson vivax infections when administered for eight weeks. It cured 90% of infections, yet did not produce clinically demonstrable haemolysis in primaquine-sensitive adult males with major expression of the haemolytic trait.

INTRODUCTION

Since 1945 Alving and his associates have conducted intensive investigations of the toxicity of newly synthesized 8-aminoquinolines, and of their causal prophylactic and curative (anti-relapse) activity in severe, mosquito-induced Chesson vivax malarial infections of inmate volunteers at the University of Chicago–Army Medical Research Unit in the Illinois State (Stateville) Penitentiary. The Chesson south-west Pacific strain, most likely of New Guinea origin, has been used in the therapeutic trials because it presents a very severe, possibly the most severe, challenge to radical cure by 8-

* From the University of Chicago—Army Malaria Research Unit. The investigations have been supported in major part by the Research and Development Command, Office of the Surgeon General, Department of the Army, under contract with the Department of Medicine, University of Chicago (current contract No. DA-49-007-MD-566).

[1] Professor of Medicine, Department of Medicine, University of Chicago, Chicago, Ill., USA.

[2] Captain, Medical Corps, United States Army.

[3] Research Assistant, Department of Medicine, University of Chicago, Chicago, Ill., USA.

[4] Senior Assistant Surgeon, United States Public Health Service.

[5] Research Associate (Assistant Professor), Department of Medicine, University of Chicago, Chicago, Ill., USA.

aminoquinolines. Primaquine, 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline, the thirty-sixth drug investigated, proved to be at least three times more active against the pre-erythrocytic (Arnold et al., 1954), and four to six times more active against the late exoerythrocytic forms of this strain of *P. vivax* than pamaquine, 8-(4-diethylamino-1-methylbutylamino)-6-methoxyquinoline, but only about half as toxic (Edgcomb et al., 1950). No other 8-aminoquinoline—including pentaquine, 8-(5-isopropylaminopentylamino)-6-methoxyquinoline, and isopentaquine, 8-(4-isopropylamino-1-methylbutylamino)-6-methoxyquinoline—of the more than fifty derivatives which have been tested at Stateville, has as high a chemotherapeutic index as primaquine.

Primaquine was first used on a large scale during the Korean conflict. A single dose of 15 mg base was administered to all United States troops on their return to home during their 10-14-day trans-Pacific voyage.[1] The programme was highly successful (Archambeault, 1954) and prevented the reintroduction of malaria into the United States of America. Only about half a dozen haemolytic reactions were reported during the return of over 250 000 troops, 10% of whom were Negroes, and none was severe.

Fifteen milligrams of primaquine base (26.5 mg of primaquine diphosphate), administered daily for 14 days, with standard three-day chloroquine therapy,[1] has proved highly effective for the radical cure of vivax malaria in most areas of the world. It is the currently recommended, or standard, anti-relapse therapy for the treatment of acute clinical attacks. The relapse rate after treatment of acute attacks of Korean vivax was less than 1% (Alving et al., 1953).

Extensive experience has established that 15 mg of primaquine base may be administered daily with safety under minimal medical supervision. This daily dose of primaquine, however, is much less effective for radical cure of vivax infections acquired in the New Guinea area of the south-west Pacific. The relapse rate in severe infections of non-immune individuals may exceed 30% (Cooper et al., 1953). The daily dose of primaquine would have to be doubled to be effective in the prevention of relapses of infections, but more than 15 mg of base cannot be administered daily without the danger of precipitating an acute haemolytic crisis in a high percentage of Negroes (Hockwald et al., 1953) and many other racial or ethnic groups, owing to a genetically transmitted abnormality of their erythrocytes (Dern et al., 1954; Carson et al., 1956; Browne, 1957).

Extensive biochemical investigations concerning the primaquine type of haemolysis, which can be precipitated by many other therapeutic agents (Dern et al., 1955), have been conducted at the University of Chicago–Army Medical Research Unit since 1950. Controlled clinical investigations exploring the possibility that the haemolytic action of primaquine might be divorced, at least in part, from its unique antimalarial actions[2] have also been conducted. The encouraging results obtained by administering 30 mg of primaquine base, together with the standard suppressive dose of chloroquine,[1] once each week for 15 weeks, which reduced the relapse rate from 90% in chloroquine-treated controls to 20% (Arnold et al., 1954), stimulated expansion of these investigations. Alving, Rucker et al. (1958) reported at the Sixth International Congresses for Tropical Medicine and Malaria, on the basis of preliminary studies, that 60 mg of primaquine base (approximately 0.4 mg primaquine base per pound of body-weight) resulted in radical cure in all but two of 44 experimental subjects, when it was administered once each week, together with 300 mg of chloroquine base, for eight weeks. All eight control subjects bitten by the same ten heavily infected *A. quadrimaculatus* mosquitos relapsed after treat-

[1] 300 mg of chloroquine base (500 mg chloroquine diphosphate), the standard regimen for the supression of malaria in the field, were also administered each week during the return of US military personnel.

A quick-acting blood schizontocide, preferably chloroquine or one of its congeners, should always be administered concurrently with primaquine because the action of primaquine against the asexual erythrocytic forms of *P. vivax* is erratic (Alving—unpublished observations), and it is almost completely inactive against the blood schizonts of *P. falciparum* (Arnold et al., 1955). Chloroquine potentiates primaquine (Alving et al., 1955). The standard chloroquine regimen for the treatment of acute clinical attacks in all human malarias is an initial dose of 600 mg of chloroquine base, followed in six hours by 300 mg, which is thereafter repeated on two successive days. The total 3-day therapy consists of 1.5 g of the base (2.5 g of the diphosphate salt).

[2] Primaquine is more active against the youngest (cryptozoite) than against the older and mature (metacryptozoite) pre-erythrocytic forms of *P. vivax*, and may even have some activity against the sporozoites. Marked true causal prophylactic action can be demonstrated in the Chesson strain by the administration of a single large dose of primaquine as early as 12 hours before or as late as 12 hours after infection of volunteers by mosquitos, but 24 hours after infection by mosquitos the prophylactic action of primaquine is considerably less and remains diminished for the duration of the prepatent period (Alving, Rucker et al., 1958; Alving et al.—unpublished observations).

Primaquine is very much more active against the pre-erythrocytic forms of *P. falciparum* than it is against the primary tissue forms of *P. vivax* (Arnold et al., 1955). It has both gametocytocidal and sporontocidal activity in *falciparum* infections (Young, 1958).

ment with eight weekly doses of chloroquine. The toxicity of the combined therapeutic regimen was negligible. They predicted that 45 mg of primaquine base, combined with the standard suppressive dose of a 4-aminoquinoline, would be effective for the radical cure of most naturally acquired vivax infections.

This interim report presents the current results of these therapeutic studies, the results of other weekly primaquine-chloroquine regimens, and preliminary results of investigations on the comparative therapeutic effectiveness and toxicity of primaquine and CN-1115 (quinocide, WIN-10,448), 8-(4-amino-4-methylbutylamino)-6-methoxyquinoline. The present knowledge of the mechanism of the primaquine type of drug-induced heamolysis also is briefly summarized.

PRIMAQUINE SENSITIVITY

For many years it has been known that pamaquine may precipitate haemolytic crises of such severity, particularly in coloured peoples, that the reactions have been mistakenly diagnosed as blackwater fever. With the development of primaquine, basic investigations of its haemolytic action were undertaken at Statevill Penitentiary. It was found that an acute haemolytic anaemia occurred in about 10%-15% of adult American Negro males during the ingestion of 30 mg of primaquine base daily, twice the dose which is now recommended for radical cure of vivax malaria.

The discovery by Dern and others (1954) that gross intravascular haemolysis occurs only in individuals whose erythrocytes have an intrinsic defect led to further studies concerning enzymatic and other biochemical abnormalities in primaquine-sensitive erythrocytes by workers at the University of Chicago and many other investigators in the USA, Israel, Italy, Germany and elsewhere. As a result, many important features of the inborn error in metabolism, originally designated " primaquine-sensitivity " and " primaquine type of drug-induced haemolysis ", have been delineated. Recently, the name " glucose-6-phosphate dehydrogenase (G-6-PD) deficiency " has also been applied to this disorder. The deficiency of G-6-PD is the most characteristic and severe abnormality which has been uncovered in primaquine-sensitive cells to date (Carson et al., 1956). It correlates better with the phenotype of drug-induced haemolysis than any other single intracellular erythrocytic biochemical defect now known. Several facts, however, suggest that the deficiency of G-6-PD is the expression of a more fundamental, but as yet unknown, abnormality which is directly determined by a mutant gene. Until the enzymatic abnormality at the gene level is known, or until the mechanism of haemolysis is fully understood, it seems preferable to us to identify the disorder by either of the older names, which have historical precedent for their use, though it is now known that primaquine-sensitive individuals are susceptible to haemolysis by many other drugs and some vegetable foods.

American Negro males who exhibit full expression of the trait were shown by Dern and his co-workers (1955) to be sensitive to many other drugs—e.g., sulfonamides—which do not produce haemolysis in non-sensitive persons, and that they are also very much more sensitive to some drugs—e.g., acetanilide and sulfones—which may cause only mild, clinically insignificant haemolysis in persons who do not have the abnormality in their erythrocytes (Desforges et al., 1959; Kellermeyer and co-workers—unpublished observations). It was soon thereafter established by Sansone & Segni (1958) and Larizza et al. (1958) in Italy, by Szeinberg, Sheba & Adam (1958a; 1958b) in Israel, and by Zinkham and others (1958) in the United States of America that haemolysis caused by the ingestion of broad, or fava, beans (*Vicia faba*), raw or partially cooked, or even by the inhalation of pollen or volatile products of the fava plant, occurs only in persons who have the same genetic pattern of biochemical and enzymatic abnormalities of the blood as is found in primaquine-sensitivity. Favism, however, apparently requires an additional extra-erythrocytic factor, probably allergic or metabolic, for its induction, because some primaquine-sensitive individuals can eat fava beans without precipitation of a haemolytic crisis. Haemolysis from fava beans may be extremely variable in a person's life and the first attack may not occur until very late in life (Brunetti—personal communication).

The growing list of drugs which, under some conditions, can precipitate haemolysis in primaquine-sensitive individuals includes several nitrofurans, naphthalene (moth balls), certain vitamin K derivatives, acetanilide, para-aminosalicylic acid, acetophenetidine, sulfanilamide, salicylazosulfapyridine (Azulfidine), and sulfamethoxypyridizine (Kynex), to name but a few. Fortunately, many of these drugs produce only mild, clinically insignificant haemolysis unless unusual factors such as severe liver disease, which may alter their metabolic degra-

dation, or renal insufficiency cause them to be retained in abnormally high concentration in the body; or unless the sensitivity to drug-induced haemolysis of an individual is enhanced by certain severe systemic diseases or concurrent bacterial or virus infection. In addition, other drugs may potentiate the haemolytic effect of primaquine when given concurrently, e.g., mepacrine (Brewer—unpublished observations).[1]

Genetic transmission of primaquine sensitivity

The inborn error of metabolism is characterized by susceptibility to haemolysis with primaquine and related noxious agents and, with rare exceptions, appears to be inherited by a sex-linked gene of intermediate dominance (Browne, 1957; Childs et al., 1958; Gross et al., 1958; Szeinberg, Sheba & Adam, 1958a) and variable expression. Complete expression of the genetic defect is common in affected males (presumably hemizygotes). Most affected females (presumably heterozygotes) show intermediate expression of the phenotype (haemolysis) (Kellermeyer, Brewer and co-workers—unpublished observations). They have less severe and more variable enzymatic abnormalities in their erythrocytes and experience milder haemolysis than do affected males. Caucasians (among them Sardinians and Sephardic Jews) with full expression of the trait, have more severe deficiency of G-6-PD than do hemizygous American Negroes, or the rare homozygous females (Marks & Gross, 1959; Szeinberg et al., 1960).

Extensive population studies have demonstrated that primaquine-sensitivity is widespread. It affects between 10% and 15% of American Negroes. It is particularly high among the natives of Sardinia (Larizza et al., 1958). It has been reported to be prevalent in certain areas of India, and occurs in 40% of some Sephardic or Oriental Jews, but is uncommonly found in Ashkenazy or European Jews (Szeinberg, Asher & Sheba, 1958). The frequency of the defect has been reported to be very high in some parts of Africa.

The distribution of the trait around the world follows a band on each side of the equator roughly corresponding to the distribution of malaria, particularly infections due to *P. falciparum*. Motulsky and others (1959, 1960) have suggested that primaquine-sensitive individuals may be more resistant to infection by this species. This interesting hypothesis can not yet be adequately evaluated.

The haemolytic state

It was shown in the early investigations of Beutler, Dern & Alving (1954a) that hypersensitivity and immune mechanisms are not essential for the initiation of the haemolytic crisis. By standard clinical methods haematological abnormalities can be demonstrated prior to drug administration. The susceptibility is not associated with any known abnormal properties of haemoglobin. It has recently been demonstrated by Kellermeyer, Brewer and their co-workers (unpublished observations) that primaquine-sensitive erythrocytes have a somewhat shorter life-span than do those of non-sensitive individuals before drug administration. They also have slightly increased resistance to hypotonic lysis (decreased osmotic fragility), which can only be demonstrated by special techniques (Tarlov et al.—unpublished observations). Thus, primaquine-sensitive individuals, though asymptomatic, can nevertheless technically be considered to have "chronic haemolysis without anaemia" even prior to the administration of this drug or other noxious agents.[2]

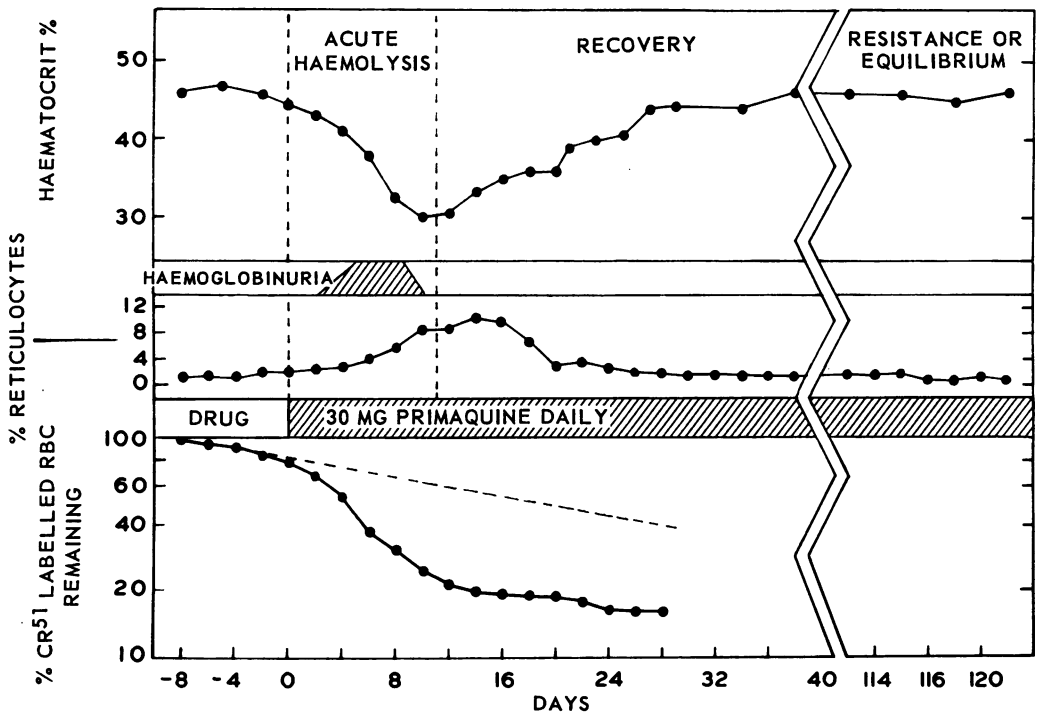
The administration of a standard dose of 30 mg of primaquine base daily to a sensitive but otherwise healthy Negro male volunteer results in a self-limited haemolysis beginning in three to four days and lasting about seven days (Fig. 1). Recovery with reticulocytosis follows even if administration of primaquine is continued, because the haemolysis is a function of cell age (Beutler et al., 1954b). Loss of activity of one or more enzymes with the aging of the cells may explain the increased vulnerability of the older erythrocytes. Marks & Gross (1959)

[1] The concurrent administration of mepacrine with primaquine impedes the degradation of primaquine—demethylation of the methoxy group in the 6- position of the quinoline nucleus—a process which is TPNH-dependent (Brodie et al., 1958). Mepacrine may interfere with the generation of TPNH by G-6-PD because, according to Haas (1944), it competes with this enzyme for its substrate, glucose-6-phosphate. Thus, the potentiation of the haemolytic action of primaquine and the increase of the undegraded, or only partially degraded, primaquine in the blood when mepacrine is administered with it, may both be due to the adverse effect of mepacrine on the activity of G-6-PD. The two phenomena may, therefore, both occur as a result of the deficiency in generation of TPNH and have no causal relationship to each other. This possibility is now being tested in clinical investigations by workers at the University of Chicago.

[2] Certain cases of congenital non-spherocytic haemolytic anaemia characterized by similar biochemical abnormalities of greater degree (Zinkham & Lenhardt, 1959) may represent a more intense manifestation of the same abnormality.

FIG. 1

CLINICAL COURSE OF PRIMAQUINE HAEMOLYSIS, SHOWN BY COMPOSITE RESULTS FROM THREE SENSITIVE MALES WITH MAJOR EXPRESSION OF THE GENETIC DEFECT



The haemolysis is self-limited even though the standard challenge dose of 30 mg daily is continued, because it stimulates erythropoiesis by the bone marrow. Reticolucytes and younger erythrocytes are relatively more resistant to haemolysis by the oxidized degradation products of primaquine than are the older cells. If the daily dose of primaquine is excessively great, chronic haemolytic anaemia results, unless the medication is terminated.

have shown that the fractions of erythrocytes of relatively young mean cell age have higher G-6-PD activity than the fractions of older mean cell age. The young cells remaining after an acute haemolysis and new erythrocytes which replace the destroyed cells are only relatively resistant to further haemolysis, however, since a second acute haemolysis can be induced by increasing the dose of primaquine (Kellermeyer et al., 1959).[1]

The first biochemical abnormality in primaquine-sensitive cells, a diminished content of reduced glutathione (GSH), was found by Beutler and his co-workers (1955). This finding was of particular interest because it had previously been shown that GSH protects erythrocytes from destruction by a variety of physical and chemical agents.

Investigations into the cause of the low glutathione content of the erythrocytes led to the discovery of the primary enzyme deficiency, that of G-6-PD, by Carson and his associates (1956). Most of the glutathione in erythrocytes exists in the reduced form (GSH), which is in equilibrium with the oxidized form (GSSG). A specific enzyme, glutathione reductase, reduces GSSG to GSH. The preferred co-enzyme is reduced triphosphopyridine nucleotide (TPNH). In the mature erythrocyte, the reduction of the oxidized form of triphosphopyridine nucleotide (TPN) to TPNH requires the oxidation of glucose *via* the pentose phosphate pathway.[2]

[1] The relative haemolytic effect of various drugs in sensitive individuals can be shown by the comparison of erythrocyte Cr^{51} survival curves with those obtained using a standard dose of primaquine or, preferably, by labelling the erythrocytes with diisopropyl-fluorophosphate^{32}. The intrinsic haemolytic action of any drug can be determined safely by he transfusion of small amounts of labelled blood from a donor known to be primaquine-sensitive into a non-sensitive recipient, who is then given the drug to be tested.

[2] See figure in the article by Brewer et al. on page 639 of this issue.

The first enzyme in this pathway is G-6-PD. The deficiency of TPNH and subsequent failure of glutathione reductase to generate GSH in sensitive cells may be explained by the severe deficiency of G-6-PD in these cells (Carson et al., 1956). TPNH is essential for important biosynthetic processes and in the degradation of drugs. *In vitro* studies of highly purified preparations of G-6-PD have failed to reveal qualitative differences between this enzyme of normal and sensitive erythrocytes (Kirkman, 1959; Marks, 1960). Carson et al. (1959) and Carson & Alving (1959) have hypothesized that the key to the mechanism of primaquine haemolysis, and probably of cellular aging, may be directly related to the binding of G-6-PD to TPN, its obligatory co-enzyme, to its stabilization by TPN, or to factors which protect the pyridine nucleotide from destruction by the pyridine nucleotidase activity of the stroma in the erythrocytes. Rimon et al. (1960) have suggested that the decreased activity of G-6-PD in sensitive erythrocytes may be explained by the absence of an activator for this enzyme in the stroma of sensitive cells.

Sensitive erythrocytes have a diminished capacity to reduce methaemoglobin by TPN-dependent methaemoglobin reductase.[1] Nevertheless, primaquine-sensitive individuals usually experience less methaemoglobinaemia than do non-sensitive subjects during haemolysis, suggesting that the older cells, which are the first to be lysed, also have a greater defect in their ability to reduce methaemoglobin than do younger cells (Kellermeyer et al.—unpublished observations).

Recent observations that primaquine sensitivity is characterized by a low rate of glycine incorporation into the GSH of erythrocytes *in vitro* (Szeinberg et al., 1959) and by characteristic *in vivo* changes in the stroma lipids during haemolysis (Tarlov et al.—unpublished observations) may reflect the importance of TPNH in maintaining the normal integrity of the erythrocytes.

A second enzyme deficiency, a decrease in catalase activity, has recently been demonstrated by Tarlov & Kellermeyer (1959). The deficiency, however, is less severe than that of G-6-PD and is not found in the erythrocytes of all individuals who manifest intermediate expression of the trait (Tarlov et al., 1960). Therefore, it probably does not represent the primary intracellular enzymatic defect. Two enzymes, glutathione reductase (Schrier et al., 1958) and probably aldolase (Schrier et al., 1959), have increased activity in sensitive cells, which has been interpreted as compensatory to the decrease of G-6-PD.

Measurement of the biochemical abnormalities during haemolysis has revealed only the following changes to occur: an initial fall of reduced glutathione and of catalase activity, followed by a transient rise in G-6-PD and GSH activity during reticulocytosis. Glutathione and G-6-PD thereafter return rapidly to pre-drug values (Flanagan et al., 1958); the fall of catalase, which is initiated by drug administration, is not fully repaired during the remaining life-span of the erythrocyte (Tarlov et al., 1960).

MITIGATION OF PRIMAQUINE TOXICITY BY ADMINISTRATION OF THE DRUG AT WEEKLY INTERVALS

Several facts suggested the possibility that the toxicity of primaquine could be reduced, by its weekly administration, without loss of the therapeutic effectiveness of this drug. For example, primaquine-sensitive individuals quickly become resistant to haemolysis after an acture haemolytic episode, but the parasites do not readily develop resistance to its action against the tissue schizonts.

The migration of primaquine toxicity (haemolysis) by intermittent weekly therapy is demonstrated in Fig. 2, which also shows that the severity of haemolysis is dependent on dosage. A dose of 60 mg of primaquine base administered weekly is less toxic than 15 mg of primaquine base administered daily; and 45 mg primaquine base administered weekly are practically without demonstrable toxicity. On the basis of these findings, it is concluded that 60 mg primaquine base (0.4 mg primaquine base per pound of body-weight) can safely be recommended for administration to well-disciplined population groups, such as military personnel; and that 45 mg weekly (0.3 mg per pound of body-weight) may safely be used in the treatment of free populations, providing some degree of supervision of drug administration can be maintained.

THERAPEUTIC STUDIES OF PRIMAQUINE [2]

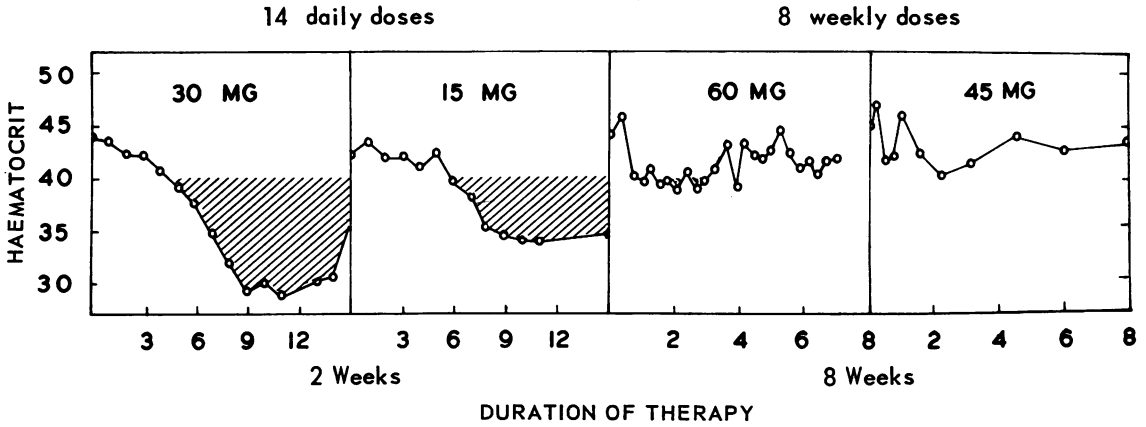
Studies comparing the therapeutic effectiveness of administering primaquine combined with chloro-

[1] The reduction of methaemoglobin to haemoglobin is discussed in more detail by Brewer et al. on page 633 of this issue.

[2] The follow-up in all therapeutic studies reported in this communication has been over 120 days, and in the majority of the studies it has been one year.

FIG. 2

COMPARATIVE HAEMOLYTIC EFFECT OF PRIMAQUINE, SHOWING MITIGATION OF HAEMOLYSIS BY INTERMITTENT WEEKLY DRUG ADMINISTRATION, THE LOWEST HAEMATOCRIT READING AFTER EACH DOSE BEING PLOTTED



The same volunteer, known to be sensitive to the administration of 30 mg of primaquine base daily, served as subject in each of the four toxicity trials, which were conducted at intervals of six months in order to allow his erythrocytes to regain their sensitivity to haemolysis by primaquine.

15 mg of primaquine daily can be administered safely, on an ambulatory basis, under minimal medical supervision. The mild self-limited haemolysis which occurs in primaquine-sensitive individuals causes no symptoms or disability.

quine (or one of its congeners) in weekly doses to that of the standard 15-mg daily-dose primaquine regimen for the radical cure of Chesson vivax malaria have been expanded sufficiently to allow tentative conclusions. Results of investigations of intermittent weekly therapy for a period of eight weeks are presented in Table 1.

When a dose of 60 mg base was employed and intermittent eight-week therapy was initiated before patency, 2.5% of infected volunteers developed patent infections; when the weekly dose was 45 mg base, 7% became patent. With intermittent weekly therapy started during the primary clinical attack, relapses occurred in 20% of infected volunteers when 60 mg was employed and in 18% when 45 mg base was employed. The pre-erythrocytic stages of *P. vivax*, therefore, appear to be somewhat more vulnerable to the action of primaquine than the late exoerythrocytic stages, but the difference in therapeutic results is not striking.

The therapeutic dosage-response curve of primaquine is of interest. Following eight weeks of intermittent combined therapy, failures were almost 60% when 30 mg base was used, but after the 45 mg dose only 10% of therapeutic failures occurred, and after 60 mg, only 6%. Considering the safety gained by the use of 45 mg, and the relatively slight

TABLE 1

RESULTS[a] OF INTERMITTENT TREATMENT OF CHESSON VIVAX MALARIA WITH 8 WEEKLY DOSES OF PRIMAQUINE

Weekly dose[b] (base)	Weekly treatment initiated on: Day of bite	7th day after bite	Day of parasitaemia	Therapeutic failures
60 mg primaquine	0/21	1/20	2/10	6%
45 mg primaquine	1/21	1/8	2/11	10%
30 mg primaquine			34/61	56%
Controls				
300 mg chloroquine	8/8		50/52	97%
15 mg primaquine daily for 14 days			16/60	27%

[a] Results are expressed as the number of patent infections or relapses over the number treated.

[b] Primaquine given concurrently with a standard dose of a 4-aminoquinoline.

gain in therapeutic effect that is obtained by the higher dose of 60 mg base per week, the lower dose would seem preferable for field use. The 45-mg regimen, employed for eight weeks, is more effective in the radical cure of vivax infections than the currently recommended therapeutic regimen (15 mg primaquine base daily for 14 days). The relapse rate was almost 30% with standard primaquine therapy in these infections.

The results of several therapeutic regimens, summarized in Table 2, indicate that, in all probability, the regimen employing 45 mg of primaquine base administered weekly for only four weeks, with chloroquine, is almost as effective as the currently recommended standard daily primaquine-chloroquine therapeutic regimen. Though a dose of 30 mg of primaquine was practically without effect in the four-week trials, and was relatively ineffective when administered for eight weeks, it proved superior to 15 mg, administered daily, when the weekly dose regimen was continued for 14 or 15 weeks.

From these considerations, it is concluded that a weekly dosage schedule of 60 mg may prove useful for the radical cure of vivax infections acquired by well-disciplined population groups or for military personnel. But the regimen employing 45 mg primaquine per week appears preferable for field use

TABLE 2

SUMMARY RESULTS OF SEVERAL REGIMENS OF INTERMITTENT WEEKLY TREATMENT OF CHESSON VIVAX MALARIA WITH PRIMAQUINE

Weekly dose [a] (base)	Number therapeutic failures / Number treated		
	14-15 weekly doses	8 weekly doses	4 weekly doses
60 mg primaquine		$\frac{3}{51}$ = 6%	$\frac{6}{20}$ = 30%
45 mg primaquine		$\frac{4}{40}$ = 10%	$\frac{6}{15}$ = 40%
30 mg primaquine	$\frac{6}{30}$ = 20%	$\frac{34}{61}$ = 56%	$\frac{37}{41}$ = 90%
Controls			
300 mg chloroquine	$\frac{10}{11}$ = 91%	$\frac{58}{60}$ = 97%	$\frac{3}{3}$ = 100%
15 mg primaquine daily for 14 days	$\frac{16}{60}$ = 27%		

[a] Primaquine given concurrently with a standard dose of a 4-aminoquinoline.

in those areas of the world where strict discipline cannot be maintained.

In areas of the world where malaria is prevalent, haemolytic and blood loss anaemias due to various types of infection and parasitic intestinal infestation are not uncommon. Fortunately, these endemic haemolytic disorders decrease the danger of haemolytic crises from primaquine because they lead to a younger circulating population of erythrocytes in the blood. Field studies have been reported in which weekly doses of primaquine, even exceeding 0.5 mg base per pound of body-weight, were well tolerated by some population groups that were presumably primaquine-sensitive (Hodgkinson—personal communication).

Studies have been reported by Hodgkinson et al. (1960) that the susceptibility of primaquine-sensitive children to haemolysis by primaquine is of the same order of magnitude as that of adults on an equal drug/body-weight basis.

PRELIMINARY OBSERVATIONS ON THE RELATIVE TOXICITY AND THERAPEUTIC EFFICACY OF PRIMAQUINE AND QUINOCIDE

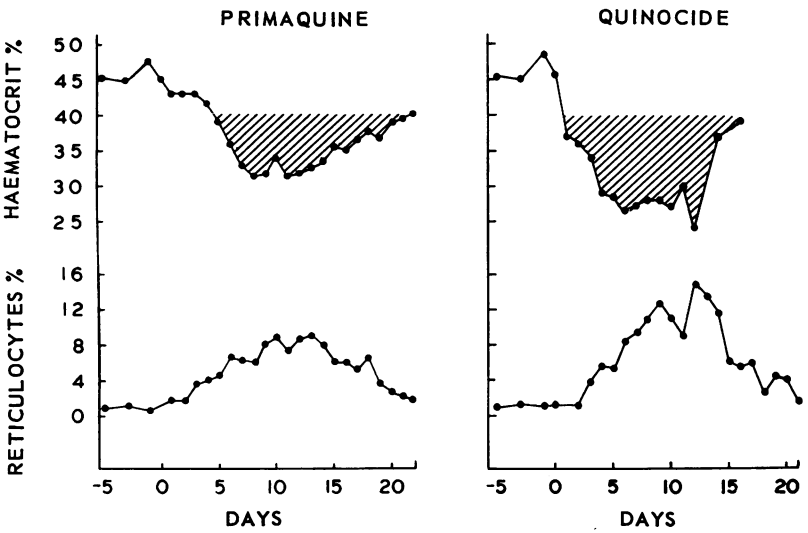
Quinocide, identified by the code number CN-1115 in the United States of America, was one of a series of isomers of primaquine which were synthesized by Elderfield and his associates in 1949 (Elderfield et al., 1955). It did not receive immediate trial in human malarial infections because tests on rhesus monkeys indicated that, both qualitatively and quantitatively, CN-1115 and pamaquine were approximately equally toxic (Elderfield—personal communication). It was synthesized by Braude & Stavrovskaya (1956) in the USSR in 1952. Its use was authorized by the Ministry of Health in 1956 (Sergiev & Yakusheva, 1956). Quinocide, or CN-1115, differs from primaquine only in the position of the methyl group of the side-chain; in CN-1115, the methyl group is in the 4- position, while in primaquine it is in the 1-position of the aliphatic side-chain.

The Russian experience has been reviewed by Bruce-Chwatt (1959).[1] This drug gave very good results when tried out in several areas and the mean number of relapses of vivax malaria was never more than 1%. The course of treatment with quinocide consists of either (1) a daily adult dose of 30 mg of the dihydrochloride for 10 days, or (2) a daily adult dose of 20 mg for 14 days. Treatment has been

[1] See also the article by A. Y. Lysenko on page 641 of this issue.—ED.

FIG. 3

COMPARATIVE HAEMOLYTIC ACTION OF PRIMAQUINE AND QUINOCIDE



Both drugs were administered in daily doses of 30 mg base for 14 days.

begun after the completion of the standard 5-day treatment of acute malaria using bigumal (proguanil) or acriquine (mepacrine). Quinocide is not given to children.

Side-effects (nausea, cyanosis, pollakisura, micro-haematuria) has, in the experience of the Soviet investigators, been seen in about 5% of patients, especially when any other antimalarials have been administered at the same time.

Relative haemolytic effect of primaquine and CN-1115

Primaquine caused less severe haemolysis after oral administration than-did CN-1115 [1] when the two drugs were compared in terms of their active base content (Fig. 3). Severe gastro-intestinal symptoms and abdominal distress were experienced by all volunteers who ingested 30 mg base of CN-1115 daily, and a dose of 45 mg base daily was barely tolerated. The ingestion of 30 mg primaquine base daily rarely produces severe symptoms.

The therapeutic effect of CN-1115 (quinocide) has been explored in Chesson vivax in two subjects, with treatment starting on the day of bite in one case and on the day of parasitaemia in the other. After the administration of 45 mg quinocide base (with 300 mg chloroquine base given concurrently) once each week for eight weeks, both subjects relapsed. The investigation of the relative chemo-therapeutic index of primaquine and CN-1115 is being expanded.

ACKNOWLEDGEMENTS

The investigations reported in this paper would not have been possible without the enthusiastic co-operation of the inmates and the administrative staff of Stateville Penitentiary, Joliet, Illinois.

The authors also wish to express their appreciation to Mrs Tertia Hall for valuable assistance in the preparation of the manuscript.

[1] CN-1115 was used as a dihydrochloride salt (78% of active base). It was furnished by Winthrop Laboratories, Inc. under the code number WIN-10, 448.

RÉSUMÉ

La sensibilité de l'organisme humain à la primaquine, qui se rencontre chez certains sujets dans les pays tropicaux et quelques autres régions du monde, est due à une défectuosité congénitale du métabolisme, caractérisée par une tendance à l'hémolyse à la suite de l'ingestion d'amino-8 quinoléines, de divers autres médicaments ou de légumineuses telles que les fèves. Le défaut des hématies est provoqué par l'insuffisance de la glucose-phosphate-6 déhydrogénase, qui affecte l'oxydation du glucose et certaines autres fonctions.

Ce défaut est déterminé par un gène lié au sexe, partiellement dominant et d'expression variable. Les hommes sont le plus fréquemment atteints. Les femmes qui héritent ce gène sont en général hétérozygotes et subissent une hémolyse moins grave que les hommes, à la suite de l'absorption de primaquine. L'hémolyse cesse d'elle-même si la dose de primaquine n'est pas excessive, car les jeunes hématies sont relativement plus résistantes. La toxicité de la primaquine est atténuée par l'administration du médicament en doses hebdomadaires — associées à la dose standard de chloroquine ou de l'un de ses équivalents. Cette posologie augmente l'efficacité du médicament dans les cures radicales d'infections à vivax. Une dose hebdomadaire de 45 mg pendant 8 semaines s'est montrée très active dans des cas d'infections graves à vivax souche Chesson. Cette dose a permis de guérir 90% des infections, sans provoquer d'hémolyse cliniquement décelable chez les sujets masculins adultes sensibles à la primaquine, chez lesquels le gène hémolytique avait sa pleine expression.

REFERENCES

Alving, A. S., Arnold, J., Hockwald, R. S., Clayman, C. B., Dern, R. J., Beutler, E. & Flanagan, C. L. (1955) *J. Lab. clin. Med.*, **46**, 301

Alving, A. S.. Arnold, J. & Robinson, D. H. (1952) *J. Amer. med. Ass.*, **149**, 1558

Alving, A. S., Hankey, D. D., Coatney, G. R., Jones, R., jr, Coker, W. G., Garrison, P. L. & Donovan, W. N. (1953) *Amer. J. trop. Med. Hyg.*, **2**, 970

Alving, A. S., Kellermeyer, R. W., Tarlov, A. R., Schrier, S. L. & Carson, P. E. (1958) *Ann. intern. Med.*, **49**, 240

Alving, A. S., Rucker, K., Flanagan, C. L., Carson, P. E., Schrier, S. L., Kellermeyer, R. W. & Tarlov, A. R. (1958) *Observations on primaquine in the cure of vivax malaria.* In: *Sixth International Congresses on Tropical Medicine and Malaria, 1958. Abstracts of papers*, Lisbon, Instituto de Medicina Tropical, p. 307

Archambeault, C. P. (1954) *J. Amer. med. Ass.*, **154**, 1411

Arnold, J., Alving, A. S., Hockwald, R. S., Clayman, C. D., Dern, R. J., Beutler, E., Flanagan, C. L. & Jeffery, G. M. (1954) *J. Lab. clin. Med.*, **44**, 429

Arnold, J., Alving, A. S., Hockwald, R. S., Clayman, C. D., Dern, R. J., Beutler, E., Flanagan, C. L. & Jeffery, G. M. (1955) *J. Lab. clin. Med.*, **46**, 391

Beutler, E., Dern, R. J. & Alving, A. S. (1954a) *J. Lab. clin. Med.*, **44**, 177

Beutler, E., Dern, R. J. & Alving, A. S. (1954b) *J. Lab. clin. Med.*, **44**, 429

Beutler, E., Dern, R. J., Flanagan, C. L. & Alving, A. S. (1955) *J. Lab. clin. Med.*, **45**, 286

Braude, M. B. & Stavrovskaya, V. I. (1956) *Ž. obshch. Khim.*, **26**, 378

Brodie, B. B., Gillette, J. R. & La Du, B. N. (1958) *Ann. Rev. Biochem.*, **27**, 427

Browne, E. A. (1957) *Bull. Johns Hopk. Hosp.*, **101**, 11F

Bruce-Chwatt, L. J. (1959) *Bull. Wld Hlth Org.*, **21**, 737

Carson, P. E., Schrier, S. L. & Kellermeyer, R. W. (1959) *Nature (Lond.)*, **184**, 1294

Carson, P. E. & Alving, A. S. (1959) *Pharmacologist*, **1**, No. 2, 46

Carson, P. E., Flanagan, C. L., Ickes, C. E. & Alving, A. S. (1956) *Science*, **124**, 484

Childs, B., Zinkham, W., Browne, E. A., Kimbro, E. L. & Torbet, J. (1958) *Bull. Johns Hopk. Hosp.*, **102**, 21

Cooper, W. C., Myatt, A. V., Hernandez, T., Jeffery, G. M. & Coatney, G. R. (1953) *Amer. J. trop. Med. Hyg.*, **2**, 949

Dern, R. J., Beutler, E. & Alving, A. S. (1955) *J. Lab. clin. Med.*, **45**, 30

Dern, R. J. Weinstein, I. M., LeRoy, G. V., Talmadge, D. W. & Alving, A. S. (1954) *J. Lab. clin. Med.*, **43**, 303

Desforges. J. F., Thayer, W. D. & Dawson, J. P. (1959) *Amer. J. Med.*, **27**, 132

Edgcomb, J. H., Arnold, J., Yount, E. H., jr, Alving, A. S., Eichelberger, L., Jeffery, G. M., Eyles, D. & Young, M. (1950) *J. nat. Malar. Soc.*, **9**, 285

Elderfield, R. C., Caflin, E. F., Mertel, H. E., McCurdy, O. L., Mitch, R. T., Ver Nooy, C. D., Wark, B. H. & Wempen, I. (1955) *J. Amer. chem. Soc.*, **77**, 4819

Flanagan, C. L., Schrier, S. L., Carson, P. E. & Alving, A. S. (1958) *J. Lab. clin. Med.*, **51**, 600

Gross, R. T., Hurwitz, R. E. & Marks, P. A. (1958) *J. clin. Invest.*, **58**, 1176

Haas, E. (1944) *J. biol. Chem.*, **155**, 321

Hockwald, R. S., Arnold, J., Clayman, C. B. & Alving, A. S. (1952) *J. Amer. med. Ass.*, **149**, 1568

Hodgkinson, R., Courtney, K. O. & Haggerty, M. (1960) In preparation

Kellermeyer, R. W., Tarlov, A. R., Schrier, S. L., Carson, P. E. & Alving, A. S. (1959) *J. clin. Invest.*, **30**, 1016

Kirkman, H. N. (1959) *Nature (Lond.)*, **184**, 1291
Larizza, P., Brunetti, P., Grignani, F. & Ventura, S. (1958) *Haematologica*, **43**, 205
Marks, P. A. (1960) *Comments on studies on erythrocyte glucose-6-phosphate dehydrogenase deficiency*. In: Gardner, L. I., ed., *A symposium on molecular genetics and human disease* (in press)
Marks, P. A. & Gross, R. T. (1959) *J. clin. Invest.*, **38**, 2253
Motulsky, A. G. & Campbell, J. M. (1960) *Blood* (in press)
Motulsky, A. G., Kraut, J. M., Thieme, W. T. & Musto, D. F. (1959) *Clin. Res.*, **7**, 89
Rimon, A., Askenazi, I., Ramot, B. & Sheba, C. (1960) *Biochem. biophys. Res. Commun.*, **2**, 138
Sansone, G. & Segni, G. (1958) *Boll. Soc. ital. Biol. sper.*, **55**, 327
Schrier, S. L., Kellermeyer, R. W., Carson, P. E., Ickes, C. E. & Alving, A. S. (1958) *J. Lab. clin. Med.*, **52**, 109
Schrier, S. L., Kellermeyer, R. W., Carson, P. E., Ickes, C. E. & Alving, A. S. (1959) *J. Lab. clin. Med.*, **54**, 232
Sergiev, P. G. & Yakusheva, A. I. (1956) [*Malaria and the fight against it*], Moscow, Medgiz
Szeinberg, A., Adam, A., Ramot, B., Sheba, C. & Meyers, F. (1959) *Biochim. biophys. Acta*, **36**, 1959
Szeinberg, A., Asher, Y. & Sheba, C. (1958) *Blood*, **13**, 348
Szeinberg, A., Sheba, C. & Adam, A. (1958a) *Blood*, **13**, 1043
Szeinberg, A., Sheba, C. & Adam, A. (1958b) *Nature (Lond.)*, **181**, 1256
Szeinberg, A., Sheba, C., Ramot, B. & Adam, A., (1960) *Clin. Res.*, **8**, 18
Tarlov, A. R. & Kellermeyer, R. W. (1959) *Fed. Proc.*, **18**, 619
Tarlov, A. R., Kellermeyer, R. W., Carson, P. E., & Alving, A. S. (1960) *J. Lab. clin. Med.* (in press)
Young, M. D. (1958) *Chemotherapeutic agents and malaria eradication*. In: *Sixth International Congresses on Tropical Medicine and Malaria, 1958. Abstracts of papers*. Lisbon, Instituto de Medicina Tropical
Zinkham, W. H. & Lenhardt, R. E., jr (1959) *J. Pediat.*, **55**, 319
Zinkham, W. H., Lenhard, R. E., jr, & Childs, B. (1958) *Bull. Johns Hopk. Hosp.*, **102**, 169