REVIEW ARTICLE

THE CHEMOTHERAPY OF MALARIA*

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MAN is concerned with only four well-authenticated species, *Plasmodium vivax*, *P.falciparum*, *P.malariae* and *P.ovale*, of some 140 known species, of the protozoon *Plasmodium* which, transmitted by the female anopheline mosquito, are the cause of malaria. There is a high degree of specificity in host-parasite relationships for, with the exception of *Plasmodium vivax* which can produce a mild parasitaemia in chimpanzees[1], none of the human parasites has yet been transmitted to other animals.

LIFE-CYCLE OF THE MALARIA PARASITE

The life-cycle of the malaria parasite is complex and in order to make clear later the roles played by the various antimalarial drugs a brief outline is now given.

With the bite of the infected mosquito, the definitive host, sporozoites from the salivary glands of the insect are injected into the blood stream of the man, the intermediate host, (see Fig. 1). The sporozoites disappear from the peripheral blood within about 30 minutes and schizonts of a pre-erythrocytic stage may subsequently be detected in the parenchymal cells of the liver[2,3]. In the case of *P. cynomolgi*, trophozoites have been detected only one day after intravenous injection of sporozoites into monkeys[4]. The nuclei of the schizonts divide and 8 to 12 days after the mosquito bite the liver cell is ruptured and a large number of merozoites are released, some of which penetrate fresh liver cells and the cycle in the liver is thereby continued. Others invade red blood cells. The first stage—trophozoite—in the blood eventually becomes a schizont which undergoes cell division giving rise to merozoites. These are released when the red blood cell disintegrates and invade further red blood cells. At this point the temperature of the host rises and a bout of fever ensues. The period of time from the initial invasion of the erythrocytes to the release of merozoites is characteristic of the species of parasite. Thus, *P.malariae* which causes quartan malaria, has a blood cycle lasting 3 days, whilst *P.vivax* and *P.falciparum* have a 2-day repeat pattern causing benign and malignant tertian malaria respectively.

During the asexual cycle in the blood, sexual forms appear—the female macrogametocyte and the male microgametocyte. When a mosquito bites at this stage the gametocytes are taken up with the blood meal and within the insect sexual reproduction takes place. A further complicated sequence of events results finally in sporozoites finding their way into the salivary glands and there they await an opportunity to infect another human subject.

* Based on one of a series of lectures on "Chemotherapy" given at The Royal Technical College, Salford, Lancashire, during October and November, 1957.

The exo-erythrocytic cycle in the human liver continues apparently independently of the cycle in the blood and relapses in vivax malaria must be attributed thereto[5]. In the case of *P.falciparum* there appears to be no continuing phase in the liver for relapses of malignant tertian malaria are not known[6].

Evaluation of antimalarials

Because of species specificity it is not possible to use the parasites of man in the laboratory for the screening and initial evaluation of potential antimalarial drugs. Avian parasites have been widely employed in this respect and the use of *P.relictum* infections in canaries led to the discovery

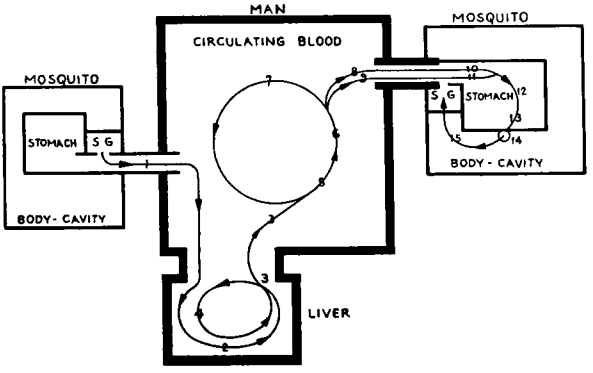


Fig. 1. Life cycle of malaria parasite:

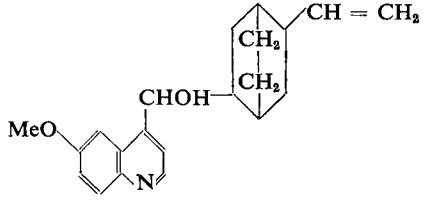
1. Sporozoites
2. Pre-erythrocytic schizonts (cryptozoites)
3. Merozoites
4. Exoerythrocytic schizonts (phanerozoite, late exo-erythrocytic forms)
5. Erythrocytic trophozoites
6. Erythrocytic schizonts
7. Erythrocytic merozoites
8. Macrogametocytes
9. Microgametocytes
10. Macrogametes
11. Microgametes
12. Zygotes
13. Ookinetes
14. Oocysts
15. Sporozoites

S.G. Salivary glands

of pamaquin[7] and mepacrine[8], *P.cathemerium* in canaries and *P.lophurae* in chicks or ducklings have received some attention and a very useful test has been based on *P.gallinaceum* infections in chicks[9,10]. In recent years the discovery of the rodent parasite *P.berghei* has enabled experiments to be carried out in mice. For further investigation of active compounds it has been usual to work either with *P.cynomolgi*, which resembles the human parasite *P.vivax*, or with *P.knowlesi* in rhesus monkeys. Final evaluation is, of course, in man himself.

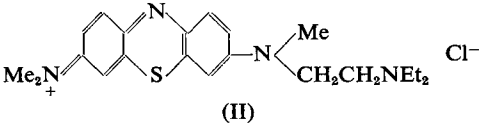
ESTABLISHED DRUGS

The earliest antimalarial drug was quinine (I), the supremacy of which was not challenged until the second world war. In the form of cinchona bark it has been in use since the seventeenth century but it was not until 1944 that its total synthesis was reported[11].



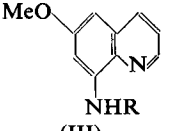
(I) Quinine

The first use of a dialkylaminoalkylamino side-chain was made by Schulemann[12] who modified the molecule of methylene blue, which had shown slight activity, to give II, which was more active than the parent



(II)

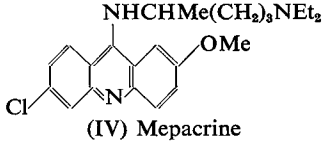
compound in avian malaria. This result led Schulemann to explore further the possibilities of the basic side-chain and its incorporation into an 8-aminoquinoline molecule provided plasmochin[13] (pamaquin, III, R = $-CH(Me)(CH_2)_3NEt_2$)



(III)

Three related compounds, pentaquin (III, R = $-(CH_2)_5NHPr^i$)[14,15], isopentaquin (III, R = $-CHMe(CH_2)_3NHPr^i$)[16,17] and primaquine (III, R = $-CHMe(CH_2)_3NH_2$) of which primaquine[18,19] seems to be the drug of choice in this series, all resulted from the American antimalarial project during the last war.

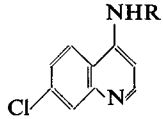
In the molecule of mepacrine (IV)[20] the basic side chain is attached to an acridine nucleus so as to form part of an extended amidine system.



(IV) Mepacrine

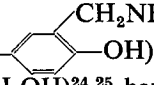
Chloroquine, (V, R = $-CHMe(CH_2)_3NEt_2$) which is structurally quite closely related to mepacrine, was first synthesised by Andersag[21] in 1941

but at that time it appears that the Germans preferred its 3-methyl derivative Sontochin.

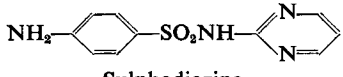


(V)

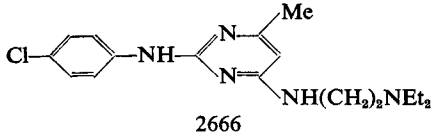
Two other 4-aminoquinolines,

amodiaquine (V, R = ) and hydroxychloroquine (V, R = —CHMe $(CH_2)_3NEtC_2H_4OH$)[24,25] have more recently become available.

When the Japanese occupied the East Indies during the second World War, supplies of quinine were denied the allied forces and an intense effort in America and Britain was made to discover a new synthetic drug. In America over 14,000 compounds were tested against avian malarias, and of these over 100 were tested in man[26]. The work in Britain led to the discovery by Curd, Davey and Rose[27,28] of proguanil (Paludrine, VI), the culmination of a sequence of steps based in the first place on pyrimidine derivatives, as outlined below (sulphadiazine was known to be active in experimental animals).



Sulphadiazine



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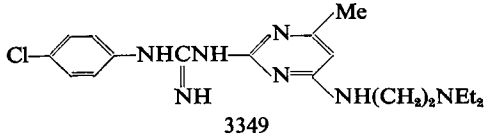

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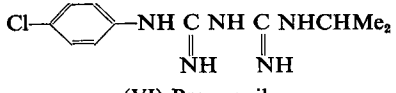
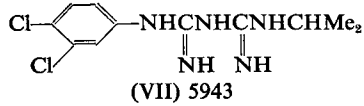


(VI) Proguanil

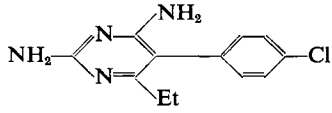
Of the many analogues of proguanil that were synthesised one deserves mention since it is now on the market (VII)[29–31].



(VII) 5943

In 1951 the pyrimidine antimalarial pyrimethamine (Daraprim, VIII) was disclosed by Russell and Hitchings[32,33]. Initial studies of 2:

4-diamino-5-aryloxypyrimidines led to the corresponding 5-aralkyl and finally to the 5-aryl compounds.



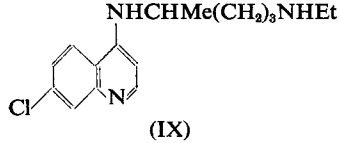
(VIII) Pyrimethamine

ANTIMALARIAL ACTION

Attack at more than one stage in the life cycle of the malaria parasite by drugs is possible. Quinine has a slight effect on primary gametocytes, otherwise it interferes only with the erythrocytic schizonts. Treatment of vivax or falciparum malaria with quinine reduces fever and parasitaemia, although its action, particularly against falciparum malaria, is unreliable. It cannot prevent, however, the occurrence of relapses in vivax malaria and it follows therefore that the exo-erythrocytic cycle in the liver is unaffected by it. Dosage regimes in man vary a great deal from drug to drug[34,35] and straight comparisons of recommended doses are difficult to make. For purposes of comparison, therefore, it is convenient, though not strictly applicable to human malaria, to quote minimum effective doses in a standard test system against *P.gallinaceum* in the chick. Quinine is fully active, that is parasitaemia is reduced to a level of less than about 1 per cent of infected red blood cells, compared with about 60 to 80 per cent in untreated controls, at a dose of about 2 mg./50 g. chick twice a day for $3\frac{1}{2}$ days[9,10]. At therapeutic doses in man it often causes mild side-reactions. It is implicated also in the aetiology of black-water fever. It is still used in cases of cerebral malaria.

Mepacrine, chloroquine and the other 4-aminoquinolines are similar in range of action to quinine—they are suppressive drugs having little or no effect on relapse rates of vivax malaria, and they produce radical cures of falciparum malaria because of the absence of late exo-erythrocytic forms with this infection. In the laboratory mepacrine is active at 2 mg. It was available in time to help the allied forces in malarious areas during the last war. Its concentration in the skin where its bright yellow colour often makes its presence conspicuous is a disadvantage. Chloroquine is colourless, much more active than quinine or mepacrine (it is fully active at 0·25 mg.) and rarely gives rise to toxic effects at therapeutic doses. Its action in the treatment of the overt attack is rapid and for this usage it is commonly regarded as the drug of choice. It is widely used as a suppressive, for, although not effective against early or late exo-erythrocytic forms, parasitaemia is suppressed before merozoites emerging from the liver can establish schizogony in the blood stream.

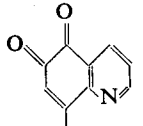
In the body chloroquine is partly converted to the compound IX[36] by loss of an ethyl group.



(IX)

The significance of the role that this compound plays in the action of chloroquine has not been elucidated. In avian malaria its activity is about the same as that of chloroquine[37] but in man when dosed orally it is poorly absorbed and is eliminated largely unchanged[36].

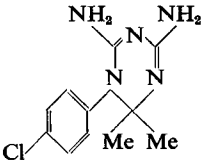
The 8-aminoquinolines are potent against erythrocytic forms in birds, for example primaquine is active at 0·02 mg. In man they were disappointing in their effect on the asexual forms in the blood and are therefore not used in treatment of attacks of malaria. They are active against both early and late exo-erythrocytic forms. Their toxicity, however precludes their routine use in causal prophylaxis but because their action against late exo-erythrocytic forms is unique they provide the only basis, in conjunction with a suppressive drug, of radical cure of vivax malaria. This series, in which primaquine with the highest therapeutic index is the drug of choice, is the only one that has a direct action on all stages of the gametocytes. Pamaquin has been shown to be metabolised to the corresponding 5:6-quinone (X)[38], which, however, is no more active *in vivo* than the parent compound.



(X)

Proguanil, 5943 and pyrimethamine are closely alike in their actions on the various stages of the parasites; and what is written about one can be taken as applicable to the others. In chicks proguanil has a marked effect on early exo-erythrocytic forms but in man whilst its action on the early tissue forms is complete with *P.falciparum* it is not so satisfactory with *P.vivax*. It is highly active against asexual blood forms (0·25 mg.) (pyrimethamine is active at 1/60th of this dose), and could therefore be classed as a suppressive—partial causal prophylactic drug. It is rather too slow in action against schizonts in man to be recommended for treatment of the overt attack. It has an indirect action on gametocytes which although they appear to be unaffected in man are prevented from completing their part of the cycle in the mosquito and the disease-transmission chain is therefore broken. These drugs therefore are of value from the public health point of view. The main advantages of this group are their effectiveness in suppression, their lack of toxicity and their cheapness.

Proguanil is without action on plasmodia growing in tissue culture but blood from animals previously dosed with the drug is markedly effective under similar circumstances[39]. The search for a metabolite the presence of which would provide a ready explanation of these results was successful. The metabolite turned out to be a dihydrotriazine derivative (XI)[40–43].

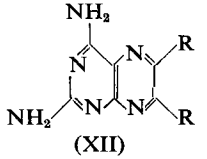


(XI)

There is no reason to doubt that it is formed by oxidation at the secondary carbon atom of the *iso*propyl group of the proguanil molecule, followed by ring-closure. The metabolite, effective at 0·025 mg., is about 10 times as active as the parent drug[41] against *P.gallinaceum* in chicks but against other experimental infections this ratio appears to be lower[44–47]. Of a large number of similar compounds synthesised[48], the most active, the corresponding 3:4-dichloro derivative (0·0025 mg.) which has been shown to be the metabolite of the corresponding diguanide 5943[41], and the proguanil metabolite have been tested clinically but although daily doses were adequate, weekly doses did not provide complete protection against *P.falciparum*[49]. In a small trial against vivax and falciparum malarias the *p*-bromo analogue also was shown to be effective[50].

Modes of action

The structure of the dihydrotriazine metabolite is of interest in relation to the structure of pyrimethamine (VIII) which has nothing more in common with that of pteroylglutamic acid than a 2-aminopyrimidine moiety. Nevertheless the two antimalarials are intimately concerned in the utilisation by plasmodia of this growth factor. Strains of plasmodia which have been made or have become resistant to pyrimethamine are in general resistant also to proguanil[51] and *vice versa*[52]. Cross resistance to proguanil and sulphadiazine[53] and to pteridine antagonists of pteroylglutamic acid[54] of the type XII also occurs.

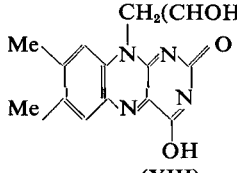


(XII)

The actions of these four types of drug against *P. gallinaceum* in chicks are all reversed by pteroylglutamic acid[55–59]. Proguanil originally said to retain its activity against *P.gallinaceum* in the presence of *p*-aminobenzoic acid[59] was later found to be antagonised by the growth factor[57,60]. A further indication of similarity of action is provided by the potentiation of the antimalarial activities of proguanil and pyrimethamine by sulphadiazine[56,61]. The antibacterial activity of the dihydrotriazines against a strain of *Streptococcus faecalis in vitro* is reversed by dihydro-, N^{10}-formyl-, and formyletetrahydro- pteroylglutamic acid (citrovorum factor) and by thymine but not by pteroylglutamic acid itself[62]. In this system, it is suggested therefore, that there is interference with the conversion of pteroylglutamic acid to citrovorum factor. The conclusion must be

drawn, although the situation is not free from contradictions (the action of proguanil against *P.berghei* has been said not to be inhibited by pteroylglutamic acid[60]), that pyrimethamine and the metabolite of proguanil interfere at an early stage with the synthesis by plasmodia of nucleic acids wherein single carbon units are incorporated into pyrimidine bases.

The details of the modes of action of other antimalarials are more obscure[63] even though a considerable amount of work which has been reviewed by Fulton[63], has been done on the effects of quinine and mepacrine in particular on the metabolism of the malaria parasite. The relationship in structure between mepacrine (IV) and riboflavine (XIII)



(XIII)

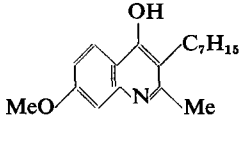
is fairly obvious and efforts to demonstrate antagonism between the two have been made[64]. The vitamin does indeed reverse the action of mepacrine, and of quinine, pamaquin and chloroquine, against *Lactobacillus casei*[65]. The effect of adenosine in producing a heart block in guinea pigs is reversed by all the drugs just mentioned[66]. The significance of these results in relation to antimalarial action has not been demonstrated.

Much has been written on the constitution of antimalarial drugs in relation to their biological activity. The basic side-chain and the heterocyclic nucleus both common to so many active compounds have often been referred to respectively as the part of the molecule necessary to bring the drug into action at the required site within the host, and the toxophoric centre[67,68]. The hypothesis that a tautomeric system as in mepacrine and the 4-aminoquinolines[69] was essential for activity, might be stretched to cover the activity of the 8-aminoquinolines now that an active metabolite, the 5:6-quinone, is known which should be capable of undergoing tautomerisation to a 6-hydroxy-5:8-quinoneimine. This sort of speculation is necessarily limited in application and can hardly be expected to lead to a true understanding of the way these drugs act.

Other active compounds

Of the drugs that have during or since the last war been extensively tested and finally rejected or are still awaiting a final assessment the following are selected for brief mention.

Endochin (XIV)[70] the best of over 100 compounds related to it has some effect on early exo-erythrocytic forms in bird malaria but has no effect in man.



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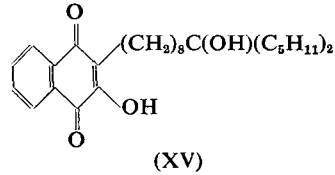


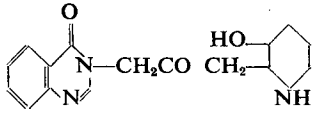
(XV)

Lapinone (XV) a member of a long series of naphthoquinone derivatives prone to degradation in the human body has been stated to possess definite action in vivax malaria after intravenous injection[71,73].

Ch'ang Shan (XVI)[74–78] shown to be identical with an alkaloid from the hydrangea[79,80] is very active in laboratory animals but low activity in man coupled with a powerful emetic action[81,82] has made the type unacceptable.

A small number of 3-amino-1:2:4-benztriazines have shown an interesting level of activity in the laboratory[83].

Many Mannich bases derived mostly from phenols have been examined[84] but only incorporation into a 4-aminoquinoline as in amodiaquine[22] or in the recently tested corresponding pyrrolidyl compound Propoquine[85] gives this type sufficient activity to be of practical importance. More recently however, the series has been extended and the compound XVII[86] has proceeded to clinical trial. It proves to be about one-third to one-half as active as chloroquine in the treatment of falciparum malaria[87].



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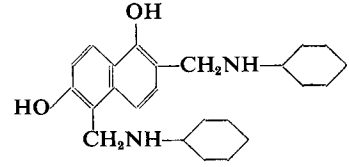

(XVII)

Another recent clinical trial has been concerned with a diaza-anthracene analogue of mepacrine, azacrine[88], which is comparable in activity with chloroquine against *P.falciparum* but slower in action against *P.-malariae*[89–91].

With the exception of the naphthoquinones all the compounds in this list are bases. Beyond this it is clearly impossible to generalise.

In conclusion it can be said that the battery of drugs for the treatment and prevention of malaria is almost as complete as possible. There are available for the treatment of the overt attack and for the radical cure of falciparum malaria, chloroquine or some other 4-aminoquinoline; for suppression, proguanil, pyrimethamine or chloroquine; for radical cure of vivax malaria, primaquine or some other 8-aminoquinoline, and for interruption of the transmission of the disease there are proguanil and pyrimethamine.

To emphasise the point it has recently been written that freedom from malaria can be practically guaranteed for any intelligent person and his family at a cost not exceeding the price of 100 cigarettes per annum[92].

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