The Effect of a Single Dose of Pyrimethamine and Primaquine in Combination upon Gametocytes and Sporogony of *Laverania falcipara* (= *Plasmodium falciparum*) in Liberia *, †

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The arrest of sporogony of *Laverania falcipara* following the administration of pyrimethamine, was first reported by Foy & Kondi.[a] Shute & Maryon [b] showed that following administration of a single dose of 25 mg of pyrimethamine 74% of mosquitos still became infected, but that these had fewer oocysts than control mosquitos and that none of the oocysts reached maturity. Jeffery et al.[c] found that pyrimethamine did not eliminate gametocytes but rendered them non-infective to mosquitos as rapidly as did primaquine. Burgess & Young [d] showed that administration of a single dose of pyrimethamine was effective within four hours, and that mosquitos fed as late as four days after medication showed only degenerate oocysts. Bray et al.[e] found, in Liberia, that a single dose of pyrimethamine (25 mg or 50 mg) prevented the development of sporogony of *L. falciparа* in *A. gambiae* in feeds made up to 28 days after medication. Retarded oocysts, which did not reach maturity, appeared in mosquitos fed as late as 14 days following medication. At the same time, Bray et al. pointed out that the formation of such oocysts with the Liberian strain of *L. falciparа* in *A. gambiae* was the exception rather than the rule as reported in the earlier studies.

Non-infectivity and disappearance of gametocytes of *L. falciparа* following a single dose of pamaquine was reported by Whitmore et al.,[f] Jerace & Giovannola [g] and Mackerras & Ercole.[h] More recently, Jeffery et al.[c] found primaquine to act similarly. Burgess & Bray [i] have reported on single-dose administration of primaquine from Liberia. In three of eight subjects sporogony proceeded to sporozoite formation in mosquitos fed one day after drug administration, and some of the mosquitos fed two to five days after medication developed degenerate oocysts but failed to show sporozoites. Gametocytes were cleared in four to eight days, with an average of 5.4 days for the series of 11 subjects.

The object of the present investigation has been to study the effect of a combination of pyrimethamine and primaquine, given as a single dose, upon gametocytes and sporogony of *L. falciparа*.

Materials and methods

All persons investigated were Liberian subjects who attended this Institute's clinic; they had all acquired the infection naturally. Their ages ranged from one year to adulthood, and their weights from 20 to 125 pounds (9-57 kg).

Only *L. falciparа* infections with gametocytes found in the peripheral blood were observed; no asexual parasites were seen. No other species of malaria parasite was seen in any of the subjects. The pyrimethamine and primaquine combination was in the form of tablets, each containing 25 mg and 20 mg (base) respectively, or a smaller tablet containing 12.5 mg and 10 mg (base) respectively.

* " While the term *Plasmodium falciparum* is in common use the choice between the generic names *Plasmodium* and *Laverania* is left open " (International Commission on Zoological Nomenclature, Opinion, No. 283). The author prefers to adjust the specific name of the parasite to the feminine generic name *Laverania*. — ED.

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[a] Foy, H. & Kondi, A. (1952) *Trans. roy. Soc. trop. Med. Hyg.*, **46**, 370.

[b] Shute, P. G. & Maryon, M. (1954) *Trans. roy. Soc. trop. Med. Hyg.*, **48**, 50.

[c] Jeffery, G. M., Young, M. D. & Eyles, D. E. (1956) *Amer. J. Hyg.*, **64**, 1.

[d] Burgess, R. W. & Young, M. D. (1959) *Bull. Wld Hlth Org.*, **20**, 37.

[e] Bray, R. S., Burgess, R. W., Fox, R. M. & Miller, M. J. (1959) *Bull. Wld Hlth Org.*, **21**, 233.

[f] Whitmore, E., Roberts, C. M. & Jantzen, W. (1930) *18th annual report of the United Fruit Company Medical Department, 1929*, p. 37.

[g] Jerace, F. & Giovannola, A. (1933) *Riv. Malar.*, **12**, 457.

[h] Mackerras, M. J. & Ercole, Q. N. (1949) *Trans. roy. Soc. trop. Med. Hyg.*, **42**, 455.

[i] See the article on page 451 of this issue.

Dosage of the combination was proportional to the patient's weight:

Weight (pounds)	(kg)	*Pyrimethamine* (mg)		*Primaquine base* (mg)
20-50	9-22	12.5	+	10
51-100	23-45	25	+	20
>100	>45	50	+	40

Gametocyte counts were made prior to medication and at daily intervals thereafter. In several instances, counts were made at more frequent intervals during the first 48 hours after medication. The elimination of gametocytes was confirmed by two negative examinations on consecutive days. At least 5000 leucocytes were counted in all cases before a film was declared negative for gametocytes. In several cases further checks were made periodically over the following month, to determine whether gametocytes had reappeared.

The morphology of gametocytes was studied, both before and after drug administration, in thick and thin films taken in an air-conditioned room and stained with Giemsa stain.

All mosquitos used in these studies were *Anopheles gambiae* from a laboratory colony maintained at this Institute. They were maintained on sugar solution at room temperature and relative humidity (72°-85°F, or 22.2°-29.5°C; 70%-100%). Control feeds were made prior to the drug administration. Subsequently mosquitos were fed at daily intervals for up to three days. Mosquitos were dissected between the seventh and tenth days for sporogony studies, and between the 11th and 14th days for the presence of sporozoites.

Results

The results of the treatment of 22 subjects with the combination of pyrimethamine and primaquine are shown in Table 1. Of the 22 cases, 19 were in children (average age 3.5 years and average weight 28 pounds, or 12.7 kg), who received the minimum dose, i.e., 12.5 mg pyrimethamine and 10 mg primaquine (base). Only one subject received the middle dose (25 mg and 20 mg respectively), and two adults received the full dosage of 50 mg pyrimethamine and 40 mg primaquine (base).

Initial gametocytaemias varied considerably, with the highest being almost 1000 times the lowest. The average initial gametocytaemia for all 22 cases (Table 2) was 483. However, Table 2 does not show the maximum gametocytaemias recorded on day 0; in six of seven cases examined gametocytaemias were, some time within the 24 hours following medication, higher than the initial number recorded. If these higher values for day 0 are taken, the initial average gametocytaemia for that day would be 514.

Clearance of the gametocytes is shown in Table 2 and the sixth column of Table 1. " Clearance time " refers to the number of days elapsing from the day of medication to the first day on which no gametocytes were found. The average clearance time for the series was five days, with a range of three to 11 days, blood films being taken at 24-hour intervals. Where checked, gametocytes did not reappear in the blood once they had been cleared.

Sporogony was studied in mosquitos fed on ten gametocyte carriers over the first three days following medication. These studies are summarized in Table 1. In all, 252 mosquitos fed following medication were dissected; 200 (from eight subjects) were gut dissections, and the rest were for sporozoites in the salivary glands.

The dissections (Table 1) showed that the drug combination disallowed sporogony. On one occasion only was a small retarded oocyst found.

Morphological studies on thin films showed that there was no detectable change in the gametocyte morphology following medication. Microgametogony proceeded normally on the first day after medication.

The combination of pyrimethamine and primaquine in a single tablet was uniformly well tolerated in all patients, and no clinical side-effects were noted or mentioned. Haematological studies [1] were carried out in one case; all findings were normal.

Discussion

The combination of pyrimethamine and primaquine, in the dosage regimen used here, completely disallowed sporogony, and hence transmission, in mosquitos fed one, two and three days after treatment of the gametocyte carrier. At these times, gametocytes are still to be found in the circulation.

The average gametocyte clearance time for the series was five days, with blood films being taken at 24-hour intervals. Twenty-one of the 22 subjects in Table 1 had initial gametocytaemias lower than 1000 per mm^3. In 18 of these the clearance times were 3-6 days, with an average of 4.1 days.

[1] Including morphology, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, fragility, icteric index and van den Bergh bilirubin reaction.

TABLE 1

EFFECT OF PYRIMETHAMINE AND PRIMAQUINE IN COMBINATION UPON GAMETOCYTES AND SPOROGONY OF *L. FALCIPARA*

Subject	Age (years) and sex	Weight (pounds)	Pyrimethamine and primaquine base (mg)	Gametocytes per mm³ at day 0 [a] (prior to drug admin.)	First day gametocytes absent	Sporogony studies					
						Day of feed [b]	Gametocytes per mm³	No. dissected	No. pos.	% pos.	Average No. oocysts (range)
1. Mw.	7 F	40	12.5 : 10	4 [c]	4 [d]						
2. M.J.	5 F	30	12.5 : 10	23	3						
3. S.D.	2½ F	26	12.5 : 10	79	3						
4. H.Ti.	2½ F	30	12.5 : 10	149 [e]	4						
5. F.G.	Adult M	125	50 : 40	32	3						
6. C.D.	9 F	50	25 : 20	360 [f]	3						
7. J.S.	3½ M	25	12.5 : 10	689	8						
8. M.D.	3½ F	27	12.5 : 10	82	4						
9. B.G.	2 M	25	12.5 : 10	64	3						
10. H.T.	4 F	30	12.5 : 10	31	5						
11. N.J.	1½ M	25	12.5 : 10	903	5						
12. I.C.	4 M	27	12.5 : 10	256	5						
13. S.B.	2½ F	25	12.5 : 10	700	5	0	700	22	17	77	29 (14-54)
						1	525	10	0	0	
						2	126	10	0	0	
14. M.G.	Adult F	100	50 : 40	193 [g]	4	0	193	10	8	80	
						1	228	5	0	0	
15. M.M.	9 F	40	12.5 : 10	162	5	0	162	40	36	90	26 (18-44)
						1	81	25	0	0	
						2	18	22	0	0	
						3	18	5	0	0	
16. H.Y.	2¼ M	27	12.5 : 10	631	5	0	631	4	4	100	63 (16-146)
						1	410	9	1 [h]	11	1 [h]
17. P.L.	1½ M	22	12.5 : 10	763	4	0	763	14	10	71	88 (32-170)
						1	171	18	0	0	
						2	124	4	0	0	
18. S.M.	6 F	42	12.5 : 10	3 895	10	0	3 895	22	2	9	10 (8-12)
						1	2 720	19	0	0	
						2	1 470	8	0	0	
19. M.B.	2½ F	25	12.5 : 10	772	11	0	772	13	13	100	85 (24-180)
						1	451	38	0	0	
20. W.O.	1 M	21	12.5 : 10	364	7	0	364	13	12	92	31 (7-87)
						1	154	3	0	0	
21. B.J.	1½ F	20	12.5 : 10	295	6	0	295	8	6	75	3 (1-7)
						1	246	8	0	0	
22. T.P.	4½ F	32	12.5: 10	185	3	0	185	25	24	96	32 (1-103)
						1	132	68	0	0	
Average				483	5.0						

[a] The day on which drug was administered was day 0.
[b] Mosquitos were fed prior to drug administration on day 0.
[c] 30 gametocytes, 6 hours after medication.
[d] Less than 1 gametocyte to 4000 leucocytes on 3rd day.
[e] 417 gametocytes, 6 hours after medication.
[f] 707 gametocytes, 6 hours after medication.
[g] 246 gametocytes, 30 hours after medication.
[h] Retarded oocyst.

TABLE 2

CLEARANCE OF GAMETOCYTAEMIA FOLLOWING ADMINISTRATION OF A SINGLE COMBINED DOSE OF PYRIMETHAMINE AND PRIMAQUINE

Day	No. of subjects	Gametocytes per mm³		
		Average	Maximum	Minimum
0	22	483 [a]	3 895	4
1	22	326	2 720	13
2	22	145	1 470	4
3	16	53	375	3
4	10 [b]	43	260	1
5	5	52	150	7
6	4	43	115	3
7	3	14	18	10
8	2	10	10	9
9	2	10	11	9
10	1	6	6	6

[a] When the maximum recorded gametocytaemia is taken, the average for Day 0 is 514.

[b] 11 subjects were positive; 10 only were examined.

The four subjects with gametocyte clearance times extending beyond six days merit separate mention. In one case (S.M., No. 18) the initial gametocytaemia was 3895 per mm³, and the long clearance time (10 days) is assumed to have been caused by the sheer load of gametocytes present. The remaining subjects in whom clearance time was prolonged all had intercurrent infections. Thus, case No. 7, J.S., (gametocytes cleared by the eight day) was diagnosed as having a mild encephalomyelitis, which may have been a sequel to vaccination, or may have followed upon a severe malignant tertian attack two days before medication with pyrimethamine and primaquine. Case No. 19, M.B., was receiving treatment for active pulmonary tuberculosis; this patient showed no gametocytes from the 11th day after medication. Lastly, case No. 26, W.O., (cleared in seven days) had a persistent leucocytosis (26 000 initially, decreasing to 14 500) with relative eosinophilia, for which no diagnosis was established.

It would appear that very high gametocytaemias take longer to clear. It would also appear that the rate of gametocyte clearance is influenced by an intercurrent infection of the host. Thus, the rate of gametocyte clearance, provided the dosage is adequate, is partially a function of the initial gametocytaemia as well as of some mechanism within the host. The three subjects cited last may be interpreted as showing impairment of the host mechanism.

Bray et al.[e] found that with a single dose of pyrimethamine alone, sporogony was arrested on the first day following medication, though gametocytes were not eliminated. On the other hand, when primaquine alone was given, Burgess & Bray [i] found that in some cases sporogony did proceed normally in mosquitos fed one day after primaquine administration. With primaquine, however, gametocytes were eliminated in an average of 5.4 days (11 subjects). The combination of pyrimethamine and primaquine was found to be highly effective: gametocytes were invariably eliminated and no infection occured in mosquitos fed on the first day after treatment. The combination thus retains the virtues of each component, and these components may be regarded as having an additive effect when used in combination.

It would seem therefore that the combination of pyrimethamine and primaquine should prove useful in mass drug administration in tropical Africa, where the aim is to sterilize gametocytes and prevent the infection of mosquitos; the use of this drug combination is advisable only in areas where no resistance to pyrimethamine has developed.

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