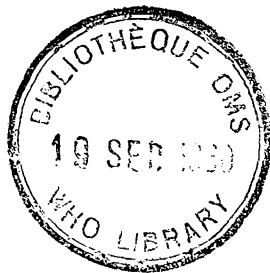


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THE EFFECT OF A SINGLE DOSE OF PRIMAQUINE UPON THE GAMETOCYTES,
GAMETOGONY AND SPOROGONY OF LAVERANIA (=PLASMODIUM) FALCIPARUM¹

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The knowledge that the 8-aminoquinolines eliminate the gametocytes of human malaria parasites is as old as the use of these drugs themselves (Muhlens, 1926). When pamaquine is given daily for three days, the gametocytes of Laverania (=Plasmodium) falciparum are eliminated in one half to three days in naturally acquired infections in Africa (British Somaliland) (Dick & Bowles, 1947).

It has also long been known that during the time of persistence of gametocytes following pamaquine treatment, Anopheles can, in a few cases, still become infected on the first and even on the second day after treatment, but not on subsequent days (Barber et al., 1929; Jerace & Giovannola, 1933).

More specifically a single dose of pamaquine will render the gametocytes of L. falciparum non-infective to Anopheles after a maximum of two days and cause their disappearance from the blood within four to eight days (Whitmore et al., 1930; Jerace & Giovannola, 1933; Mackerras & Ercole, 1949). Primaquine was found to act similarly (Jeffery, Young & Eyles, 1956) but only in one trial was a single dose used by these authors. As single dose treatment combining amodiaquine and primaquine has been used for the treatment of immigrants in some areas of Africa where malaria eradication programmes are under way (Alves, 1958), it seemed

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desirable to test the effect of single doses of primaquine upon sporogony of L. falciparum in a larger number of cases.

There appears to be some disagreement as to the site of action of the 8-aminoquinolines in regard to gametogony and sporogony. Pinto (1930) reported that pamaquine halted microgamete formation in L. falciparum. Mudrow-Reichenow (1953) reported that primaquine and pamaquine inhibited the microgamete formation of P. cathemerium five hours after administration. Jerace & Giovannola (1933) and Mackerras & Ercole (1949) agree that pamaquine does not always inhibit microgamete formation of L. falciparum at various times after administration. Mackerras & Ercole (1949) reported oökinete formation as "delayed" in feeds made nine to fifteen hours after drug administration. Attempts to obtain some more definite information on this point are also reported here.

MATERIALS AND METHODS

Colony-bred A. gambiae of a proper age were prepared for feeding by removing all food and water at least ten hours prior to applying them on the gametocyte carrier.

Test subjects with naturally-acquired L. falciparum infections included five adults and seven children between three and seven years of age.

After the control feeding on day 0, a single dose of primaquine, 15, 30 or 45 mg base (according to age) was administered to each subject. Subsequent mosquito feedings were made at intervals of one or two days up to the seventh day after treatment. Prior to each feeding gametocyte counts were made (Table I). Examination for gametocytes continued daily until these had been absent for several days.

Gut dissections were usually started on the sixth or seventh day after the blood meal. If a series of gut dissections was negative for oöcysts, later gland dissections for sporozoites were dispensed with. When gut dissections were positive, later salivary gland examinations were usually made.

In order to determine the effect of primaquine on the developing parasite in the mosquito, a malaria-free adult was dosed with 45 mg primaquine. Three hours and again four hours after treatment, mosquitos with a three-day-old infection of L. falciparum (having fed three days earlier on a gametocyte carrier) were allowed to feed on the primaquine-treated subject.

On two occasions microgamete and ookinete production was examined in mosquitos having fed on untreated and on primaquine-treated subjects in order to locate the point of action of the drug.

All incubating mosquitos were kept at room temperature (72-86°F) and a relative humidity rarely below 74 per cent., and fed on sugar-water and corn syrup.

RESULTS

Table I

The numbers of crescents per mm³ on day "0" before treatment are given in the first line of table I. Subsequently are shown succeeding counts made prior to each feeding; the column on the right indicates the number of subjects included. On day 1 - the day following treatment - the average gametocyte count was diminished very little. Later counts became progressively smaller. The first day upon which gametocytes were not found varied from four to eight days after treatment, with an average for eleven subjects of 5.4 days.

Table II and Table III

Tables II and III show the effects of the primaquine treatment as indicated by developmental failure of the parasite in the mosquito.

While, as shown in table I, the gametocyte count remained in general high on the day following treatment, there was on the other hand a considerable drop in the percentage of infected mosquitos, as may be seen from tables II and III. Only three out of eleven subjects were able to infect Anopheles (including glands) on day 1 after treatment. One was a pregnant sixteen-year-old girl who had been given 30 mg and the two others were adults who had received 45 mg each. All three

batches of mosquitos exhibited - in addition to normal oöcysts - some of the "retarded" or "disintegrated" oöcysts, the appearance of which is that of a small, abnormal or very young oöcyst; they never develop to maturity. Such oöcysts have been observed and depicted before (Shute & Maryon, 1954; Burgess & Young, 1959).

All gut infections in mosquitos fed on days 2, 3 and 4 after drug administration consisted of the above abnormal oöcysts which are known to produce no gland infections (Table III).

In order to determine the effect of primaquine on the developing parasite in the mosquito, A. gambiae were fed upon an untreated gametocyte carrier. Three days later these mosquitos were divided into three groups:

Group 1. Remained un-fed as a control.

Group 2. Was re-fed on a malaria-free subject who had received 45 mg primaquine three hours before the feeding.

Group 3. Was re-fed on the same malaria-free subject who had received 45 mg primaquine four hours before the feeding.

Results of all dissections (guts and glands) were as follows:

	Total percentage positive
Group 1 (control)	34.6
Group 2 (three hours)	38.9
Group 3 (four hours)	51.6

Gland infections were found only in groups 2 and 3.

Apparently the drug ingested with the blood during the second feeding had no adverse effect on the oöcysts.

Microgamete production appeared to proceed normally after primaquine treatment. The number of oökinetes observed in mosquitos fed before treatment and in those which fed 24 hours after treatment were approximately equal when gametocytaemias were approximately equal. However, in mosquitos fed after treatment, oökinetes were substantially smaller and differed in their reaction to

Giemsa stain. Forty-eight hours after feeding the number of oökinetes in the lumen of the gut of mosquitos fed on primaquine-treated subjects exceeded the number found in mosquitos fed prior to drug administration and these oökinetes stained pink throughout with Giemsa, no nucleus being visible. Oökinetes from mosquitos fed on untreated subjects stained normally.

DISCUSSION

A single dose of primaquine in the range of 15 mg base for children up to 45 mg base for adults slowly eliminates the gametocytes of L. falciparum. Although the time of elimination may last up to four to eight days, only on the first day after primaquine administration in a few cases were gametocytes still infective to A. gambiae and capable of producing sporozoite infections. Only three such exceptions were observed in the reported series of 12 gametocyte carriers treated with a single dose of primaquine. In none of the 12 subjects (which included a girl of 16 years, seven months pregnant, who received 30 mg base primaquine) was any toxicity or untoward side effect noted. No recrudescences of gametocytaemia were seen. Thus it can be said that a single dose of primaquine is effective in eliminating L. falciparum gametocytes and preventing infection of mosquitos and therefore is a very useful additive drug for the treatment of malaria infections in immigrant populations entering malaria-freed zones where anopheline vectors continue to be present.

The mode of action of primaquine on gametogony and sporogony appears to be similar to that recorded for pyrimethamine by Bray et al. (1959). Microgamete formation in the mosquito seemed to be normal and oökinetes appeared but these oökinetes did not find their way to the gut wall and by 48 hours were degenerating.

SUMMARY

Twelve subjects showing gametocytes of Laverania falciparum were treated with a single dose of 15, 30 or 45 mg of primaquine base, according to age. Batches of Anopheles gambiae were fed before and up to seven days after treatment.

It was found that a single dose of primaquine clears the blood of crescents in four to eight days. In five out of eight cases (see tables III and IV) no gland infections were seen in A. gambiae fed on the first day after treatment; in three cases, however, sporozoites were found. Subsequent to the first day following treatment no sporozoite infection of A. gambiae occurred in the three cases in which gland dissections were made. Small retarded oöcysts were seen occasionally. Feeding oöcyst-infected A. gambiae on a subject having received primaquine three and four hours prior to the feed had no effect upon the development of the parasite.

Following treatment, gametogony was apparently normal but oökinetes did not penetrate the gut wall, showed staining differences after 24 hours, and appeared to be dead after 48 hours.

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Table I. Gametocyte counts on first (day 0) and subsequent days of primaquine treatment

Day	Gametocytes per mm ³			Number of subjects
	High	Low	Mean	
0	2310	69	618	12
1	2296	38	475	11
2	1101	19	352	7
3	435	11	142	7
5	132	22	77	2
7	34	0	17	2

Table II. Gut infections in mosquitos fed on first (day 0) and subsequent days of primaquine treatment

Day	Guts dissected	Positive guts	Percentage positive	Number of subjects	C.I.***		
					High	Low	Mean
0	307	237**	77.2**	12	9950	156	4334
1	316	86*	27.2*	11	3820	0	467
2	185	1*	0.5*	7	3	0	0.5
3	224	13*	5.8*	7	36	0	5
5	67	1	1.5	2	2.5	0	1
7	57	0	0.0	2	0	0	0

* Oöcysts retarded and degenerate.

** Some retarded and degenerate oöcysts present in addition to normal oöcysts.

*** C.I. = Calculated Index. The calculated number of oöcysts in 100 gut dissections (= average oöcysts per infected gut x per cent. positive guts).

Table III. Gland infections in mosquitos fed on first (day 0) and subsequent days of primaquine treatment

Day	Glands dissected	Positive glands	Percentage positive	Number of subjects	C.I.**		
					High	Low	Mean
0	154	132	85.7	9	390	112	268
1	133	27	20.3	8*	311	0	78
2	22	0	0.0	2			
3	18	0	0.0	1			

* All positive glands occurred in feeds from three subjects.

** C.I. = Calculated Index, see table II. Instead of oöcyst numbers per gut the numbers of sporozoites per glands stated as plus signs are used in the C.I.

- + (1) = 1-10 sporozoites
- ++ (2) = 11-100 sporozoites
- +++ (3) = 101-1000 sporozoites
- ++++ (4) = over 1000 sporozoites

Table IV. Effect on sporogony of *L. falciiparum* in *A. gambiæ* of primaquine given on Day 0 to crescent carriers

Patient	Age in years	Primaquine dose in mg base	Crescents/ per mm	First day crescents absent	Day of feed	Number of guts dissected	Number positive	Percentage positive	Average number of oöcysts per positive gut	Percentage glands positive
(1) C.D.	4	30	1431	6	0	55	19	34.5	39.2	None diss.
			175		3	22	0	0.0	0	" "
			0		7	21	0	0.0	0	" "
(2) C.D.	16*	30	2310	8	0	24	22	91.7	31.5	85.7
			2296		1	23	23	100.0	38.2**	77.8
			435		3	43	13	30.2	1.2**	0.0
			132		5	40	1	2.5	1.0	None diss.
			34		7	36	0	0.0	0	" "
(3) S.W.	7	15	161	6	0	25	20	80.0	54.4**	None diss.
			350		1	37	8	21.6	2.5	0.0
			146		3	30	0	0.0	0	None diss.
			22		5	27	0	0.0	0	" "
			257	5	0	29	25	86.2	40.0***	None diss.
(4) L.P.	4	15	277		1	27	2	7.4	1.5	0.0
			31		3	28	0	0.0	0	None diss.
			133	4	0	23	15	65.2	2.4**	46.7
(5) S.S.	35	45	68		1	37	1	2.7	1.0	None diss.
			11		3	40	0	0.0	0	" "
			708	6	0	18	15	83.3	105.6**	91.7
(6) F.G.	30	45	568		1	14	11	78.6	2.0	54.5
			539		2	32	0	0.0	0	None diss.
			108		3	29	0	0.0	0	" "
			482	6	0	17	17	100.0	99.5**	94.7
(7) G.L.	7	15	332		1	21	5	23.8	2.3	0.0
			202		2	17	0	0.0	0	None diss.
			85		3	32	0	0.0	0	" "
(8) A.B.	3	15	1166	No data	0	21	13	61.9	41.4	80
			697		1	34	0	0.0	0.0	None diss.
			1101		2	34	0	0.0	0	" "

* At least some retarded and disintegrated oöcysts.

** Average number of oöcysts from only four dissections.

*** Pregnant seven months.

Table IV (continued)

Patient	Age in years	Primaquine dose in mg base	Crescents/ per mm ²	First day crescents absent	Day of feed	Number of guts dissected	Number positive	Percentage positive	Average number of oocysts per positive gut	Percentage glands positive
(9) N.K.	5	30	140 38 19	5	0 1 2	28 30 39	27 0 0	95.4 0.0 0.0	15.2 0 0	88.9 0.0 0.0
(10) E.J.	Ad.	45	69 39 24	5	0 1 2	32 19 10	31 1 0	96.9 5.3 0.0	13.7 1 0	100.0 0.0 0.0
(11) N.	6	30	181 88 60	4	0 1 2	11 35 25	11 0 0	100.0 0.0 0.0	70.2 0 0	71.4 None diss. "
(12) K.W.	Ad.	45	429 475 520	4	0 1 2	24 39 30	22 35 1	91.7 89.7 3.3	94.6* 6.1* 1.0	95.2 50.0 None diss.

* At least some retarded and disintegrated oocysts.