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THE APPEARANCE OF PYRIMETHAMINE RESISTANCE IN P. FALCIPARUM
FOLLOWING SELF-MEDICATION BY A RURAL COMMUNITY IN GHANA¹

by

L. J. Charles,² H. J. van der Kaay,³ H. I. Vincke³ and J. Brady³

INTRODUCTION

Adoption in Tropical Africa of the newer technique of residual house spraying for malaria eradication brought early disappointments, and later to West Africa, its quota of vector resistance problems. Only more recently have the factors contributing to poor results in imagocide control come under close scrutiny, and detailed attention to some of them has produced encouraging results in Liberia and Cameroun Republic. In the interval between these two eras, considerable effort was directed to field study of mass drug regimens which might serve as suitable complements to the house-spraying campaign. To date, the drug most commonly employed has been pyrimethamine, because of its causal prophylactic and sporontocidal action, especially against P. falciparum, the dominant malaria parasite species of the region.

With few exceptions, however, particularly when the dosage interval has been extended in an attempt to reduce distribution costs, selection has occurred of strains of P. falciparum resistant to the drug. Thus, Clyde & Shute (1954), using monthly adult doses of 100 mg pyrimethamine in Tanganyika reported resistance in P. falciparum within three months, and the same authors (1957) encountered similar resistance after five months of a weekly regimen ranging from 6 mg in infants to 50 mg in adults. In Southern Nigeria monthly adult dosage of 25 mg pyrimethamine

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² Formerly Malaria Unit, Ministry of Health and Social Welfare, Ghana, at present with WHO Malaria Advisory Team, Iran.

³ WHO staff member, Malaria Eradication Pilot Project, Ghana.

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was associated, after eight distributions, with failures to interrupt P. falciparum sporogony in A. gambiae (WHO, 1958). Ricosse, Bailly-Choumara, Adam & Hamon (1959), with fortnightly adult doses of 50 mg in the new Republique Voltaique, recorded resistance within three and four months respectively in unsprayed and DDT-sprayed sectors of a pilot malaria eradication project. More recently Archibald (1960), using 25 mg monthly adult doses in an unsprayed Northern Nigerian village encountered pyrimethamine resistance during the fourth to sixth months of supervised distribution.

One of us (Vincke, 1954) had recorded encouraging results in sprayed areas of high altitude in Katanga (Congo Republic) where short courses of weekly pyrimethamine tablets were distributed under the supervision of village chiefs.

In an attempt both to adhere to the weekly adult 25 mg pyrimethamine generally recommended for personal prophylaxis (Rollo, 1955) and to eliminate the need for official tablet distributors, a field trial was carried out with the drug in the Volta Region of eastern Ghana. Preliminary results obtained have been reported by one of us (Charles, 1960) but a fuller account is set out in this paper.

METHODS

The trial formed part of the preparatory phase within the area of a malaria eradication pilot project embracing a population of 700 000 and supported by the Ghana Government, UNICEF and WHO. The venue chosen was the small village of Akrofu Heviofe (Village I) with 721 inhabitants who had previously participated successfully in two community development projects under the stimulus, guidance and supervision of the Mass Education Division of the Department of Social Welfare and Community Development. The village continued to be serviced by that Division through a well-attended weekly class in home-craft and it was generally considered one of the more co-operative communities among the Ewe tribal settlements in the region. No prior antimalarial measures had been employed in the locality.

After the residents had been prepared by preliminary educational propaganda and a detailed nominal census of the occupants of each compound had been made, the trial was launched on 3 November 1958. Thick blood films were taken on representative

samples of all ages and the initial administration of pyrimethamine was made individually at a dosage of half a tablet (12.5 mg) to infants and toddlers aged under three years, and 25 mg to all others. Attendance was entered against the names listed on household cards.

Blood films were stained with Giemsa and examined at the laboratory, and the results of anopheles house-captures and salivary gland dissections made within ten days of the start of the trial, served as base-line entomological data.

The three ensuing weekly treatments were made, as on week 0, by individual administration, two teams each of recorder and issuer completing the task in approximately five hours. From week five, attendance of the entire population was discontinued. A responsible member of each household was required, on the appointed day, to collect at the distribution centre the number of tablets needed for the family in accordance with the ages and reported presence of household members. Tablets were not issued for individuals who were expected to be away for more than a week from the village. For those present, reliance was placed on self-administration of the drug at home.

After three weeks' direct management of this distribution system responsibility for its maintenance was delegated on week eight to three teams each of two volunteers drawn from the three clans constituting the core of the social structure of the village.

Because gland-positive A. gambiae were still being encountered at Village I by week nine it was considered desirable to safeguard against probable anopheline incursion from a neighbouring untreated village one mile away on a second-class motor road. On 14 January 1959, therefore, 10 weeks after the start of the trial, this second village, Akrofu Agove (Village II) with a population of 356 was surveyed and placed on the same weekly pyrimethamine regimen. Arrangements similar to those at Village I were made for local issue of tablets, and on that voluntary basis the trial was continued until October 1959 (weeks 49 and 38 respectively for Villages I and II), with periodic supervisory visits by official personnel.

RESULTS

Before treatment

At both villages the pre-treatment malaria picture approximated that of hyper-endemicity with spleen and parasite rates of 55 and 72 per cent. respectively among the age-group 2-9 years. The combined data for the two villages showed parasite rates rising from 31 per cent. in infants under 1 year to 73 per cent. in the age-group 5-9 and falling off to 23 per cent. in adults. Only P. falciparum and P. malariae were identified.

Dissections of house-caught anopheles made within 10 days of the start of the drug trial showed sporozoite rates of 7.3 and 14.7 per cent. respectively in 248 A. gambiae and 51 A. funestus - the only vectors encountered.

After treatment

Early results. At Village I, 168 blood films were examined from among those children aged 0-14 years who were originally blood-positive on week 0. Although 11 (6.5 per cent.) were again positive, only one - that of a 10-year old boy - contained trophozoites of P. falciparum. The others showed only crescents carried over from week 0 (Table 2).

Subsequent blood surveys on weeks 16 and 22 showed parasite rates of 5.5 and 3.2 per cent. respectively on samples of 162 and 219 children aged 0-14 years (Table 2). The positives of week 16 were not investigated, but of seven encountered on week 22 only three of those with P. falciparum rings were permanent residents of the village. After two consecutive directly supervised weekly tablet administrations at the appropriate dosage, they were re-examined and found free from asexual forms of P. falciparum.

At Village II, the week 0 parasite rate of 55.3 per cent. among the age-group 0-14 years had fallen to 7.4 per cent. by week 6 (Table 2). Of the four positives, all of whom showed P. falciparum trophozoites, one was a permanent resident who had not left the village during the six-week period and who, on week 0, was positive for crescents only. The other three children, aged 7 and 10 years, were new arrivals of 3, 2 and 1 week's standing, but their history of tablet ingestion and subsequent blood film results were not investigated at the time.

Late results. A parasitological evaluation was made on 13-14 July 1959, i.e. on weeks 37 and 26 for Villages I and II respectively.

At Village I positive blood films were found in all ages with a crude parasite rate of 18.8 per cent. on 362 examinations. Of the 68 positives, 51 had P. falciparum only, 16 P. malariae (singly or mixed) and one P. ovale.

At Village II results were comparable, with a crude parasite rate of 21.4 per cent. contributed to by all but the age-group over 20 years. Only P. falciparum was identified in the 31 positive films. At the two villages the rates for children aged 0-14 years were 25.3 and 28.3 per cent. respectively (Table 2).

This sharp increase in parasite rate from 3.2 to 25.3 at Village I in the interval between weeks 22 and 37 warranted further investigation, and this was undertaken a fortnight later. As many as possible of the original positives of 13-14 July were traced at both villages; thick blood films were taken (day 0); the appropriate dose of pyrimethamine was administered under direct supervision; and following films were made on days 3 and 7. Of 63 persons thus re-examined on day 0, only 32 were still found with parasitaemia, and among these, six continued to show P. falciparum rings on day 3, and four on day 7. These four were re-treated with the routine tablet dosage on day 7, and although three of the infections survived for an additional three days, they were all cleared by the sixth day after the second treatment.

PYRIMETHAMINE RESISTANCE IN P. FALCIPARUM

The trial was continued without modification for a further period of three months, and the results were again evaluated on 5-6 October 1959, i.e. on weeks 49 and 38 respectively for Villages I and II. On this occasion all available laboratory personnel were deployed so that the blood film examinations could be completed within three days.

The results showed that the crude parasite rate at both villages had risen to 49 per cent. but since there was little difference between age specific rates at Villages I and II, the results have been combined for presentation in the second half of Table I, and for purposes of comparison, the rates for children aged 0-14 years -

62.1 at Village I and 57.3 per cent. at Village II - have been included in Table 2. In this survey all three malaria parasite species normally encountered in Ghana were found, with P. falciparum predominating.

On the fourth day after this survey, efforts were made to trace the blood-positives of all ages from Village II and those of school age from Village I. Collection of new films (day 0) was followed by administration of the routine dose of pyrimethamine under direct supervision, and the follow-up blood films were taken on days 3 and 6 from those showing trophozoites on day 0. The P. malariae and P. ovale infections were all cleared by day 3, but over 70 per cent. of P. falciparum infections survived to day 6 (Table 3).

This evidence of P. falciparum resistance to pyrimethamine was confirmed in a small number of children, selected for persistent trophozoites, by the administration of larger single doses of pyrimethamine. Follow-up blood film examinations showed that in the majority of these cases asexual forms of P. falciparum survived for six days after dosages of the drug equivalent to twice the routine doses hitherto employed (Table 3).

Finally, separate batches of laboratory-reared A. gambiae were fed immediately before, and three days after pyrimethamine treatment of children positive for P. falciparum gametocytes. The mosquitos were kept in the insectary (at 25-28.5°C and R.H. 80 per cent.) and dissected 13 days after the blood meals. Examination for oocysts was made only on specimens showing no sporozoites, and the combined results are set out in Table 4. Of 42 anopheles which survived the blood meal taken three days after pyrimethamine treatment, 22 were positive for sporozoites. One additional specimen had five mature oocysts which released active sporozoites when squashed. In the two untreated children who served as controls, sporozoites were also found after the feedings of days 0 and 3 respectively.

While these several observations were being made, routine mosquito house captures were continued at Village I. Salivary gland dissections showed that the sporozoite rates in A. gambiae and A. funestus fell sharply from the initial 7.3 and 14.7 per cent. to 2.5 and 4.3 per cent. respectively by week 4 of the drug trial. Without exception, however, the combined sporozoite rate for the two species remained above

one per cent. during each subsequent four-week period (although the A. gambiae population was negative during weeks 13-20), and had risen to 7.7 per cent. in October 1959 when the present observations were discontinued.

DISCUSSION

There was no doubt of the high degree of enthusiasm which the trial generated among the villagers. For, from week 5 onwards when family distribution was made only to one member of each household, the weekly proceedings were invariably completed by 0730 hours and did not, therefore, interfere unduly with departures for the farms.

Susceptibility of the local P. falciparum strain to pyrimethamine was demonstrated by the results of week 4 when all but one of a large sample of week-0 positive children at Village I were shown to be free of asexual forms. However, no firm conclusion could be drawn on the status of the four positives encountered on week 6 at Village II. They had been recorded as having taken one or more weekly treatments, but in the absence of investigation into their history of tablet ingestion and follow-up blood picture, the possibility could not be wholly excluded that one or more of them originally harboured a strain of P. falciparum initially refractory to the drug.

Even as late as week 22 at Village I, however, investigation of three trophozoite-positive indigenous children showed apparently normal response to standard weekly doses of pyrimethamine. By week 37, however, increased tolerance in P. falciparum was evident not only at Village I, but also at Village II which was then in its twenty-sixth week of treatment; and by week 49, selection of a pyrimethamine-resistant strain of P. falciparum was confirmed.

There was evidence that this selection was at least associated with irregular ingestion of the weekly tablets. This was supported by the re-appearance of P. malariae singly and in mixed infections by week 37, and the appearance of P. ovale at that examination - both of which showed normal response to supervised doses of pyrimethamine up to week 49. Furthermore, among persons positive on 13-14 July 1959, only 51 per cent. of 63 examined a fortnight later continued to show asexual P. falciparum parasitaemia. This strongly suggested that half of them had taken at least one weekly

treatment in the interim. More direct evidence of irregular medication was obtained by questioning schoolchildren on week 49 and 38 respectively in Villages I and II, when 49 per cent. of 91 admitted that their previous week's tablet had not been administered. There were suggestions that at least some of the tablets distributed were being sold or shared at villages in the general area, but no definite proof of this was obtained.

In evaluating the reasons for failure of this trial it is impossible to discount the probable effect of more frequent mass propaganda-type village gatherings in helping to maintain regular individual medication. On the other hand, such repeated efforts can hardly be considered practicable on any but a limited scale in a rural area with its scattered settlements. Nor can it be overlooked that Village I was already conditioned to community effort; health educational work was actively maintained; and satisfactory tablet distribution was made, not by strangers, but by acknowledged leading members of the village communities. Breakdown ostensibly occurred at the household where continued administration rested on individual responsibility, either because of the comparatively long period involved or the desire to extend the benefits of the free tablets to relations, friends and acquaintances in the vicinity.

SUMMARY

After initial individual administration of weekly pyrimethamine to the population of two villages in Ghana, a system of voluntary distribution to household representatives was instituted.

Early reduction in parasite rate at the first treated village was sustained for approximately 22 weeks when P. falciparum trophozoites in positive indigenes were still susceptible to the drug.

By the thirty-seventh and twenty-sixth weeks at Villages I and II, parasite rates were again on the increase; re-appearance of P. malariae and P. ovale suggested irregular self-medication, and there was evidence of enhanced tolerance to the drug in P. falciparum.

Resistance in P. falciparum to pyrimethamine was confirmed at both villages on the forty-ninth and thirty-eighth weeks respectively. Parasite rates had reverted to pre-treatment levels and asexual forms survived six days after single doses of the drug equivalent to twice the routine schedules. Supervised treatment of two gametocyte carriers failed to interrupt sporogony in A. gambiae.

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TABLE 1. COMBINED MALARIA PARASITE RATES AT TWO VILLAGES BEFORE AND AFTER A WEEKLY SELF-ADMINISTERED PYRIMETHAMINE TRIAL IN GHANA

Age-Groups (yrs)	Before treatment			After treatment*		
	Number exam.	Positive		Number exam.	Positive	
		Number	%		Number	%
0 < 1	26	8	31	25	13	52
1 < 2	31	25	81	8	7	87
2 < 5	106	76	72	59	39	66
5 < 10	132	96	73	142	85	60
10 < 15	108	65	60	61	29	47
15 < 20	76	33	43	13	8	61
20 +	381	89	23	108	23	21
TOTAL	860	392	46	416	204	49

* Weeks 49 and 38 at Villages I and II respectively.

TABLE 2. RESULTS OF PERIODIC MALARIA SURVEYS ON CHILDREN AGED 0-14 YEARS AT TWO VILLAGES BEFORE (WEEK 0) AND AFTER INITIATION OF A SELF-ADMINISTERED WEEKLY PYRIMETHAMINE FIELD TRIAL IN GHANA

	Village I (721 population)						Village II (356 population)			
	Week of treatment						Week of treatment			
	0*	4	16	22	37	49	0**	6	26	38
Number examined	300	168	217	219	233	199	103	54	106	95
Number positive	213	11	12	7	59	118	57	4	30	55
Parasite rate %	71.0	6.5	5.5	3.2	25.3	59.3	55.3	7.4	28.3	57.3

* 3 November 1958

** 14 January 1959

TABLE 3. PERSISTENCE OF P. FALCIPARUM TROPHOZOITES AFTER SUPERVISED DOSES OF PYRIMETHAMINE AT TWO VILLAGES ON WEEKS 49 AND 38 RESPECTIVELY OF A WEEKLY SELF-ADMINISTERED PYRIMETHAMINE FIELD TRIAL IN GHANA

Pyrimethamine dosage	Day after treatment				
	Day 0	Day 3		Day 6	
	Positives*	Pos./ exam.	% pos.	Pos./ exam.	% pos.
Routine**	86	55/76	72.4	52/68	76.5
Routine X 1.5	9	8/9	88.8	6/8	75.0
Routine X 2	7	6/7	85.7	6/7	85.7

* Selected for P. falciparum trophozoites.

** 0-3 years: 12.5 mg; over 3 years: 25 mg.

TABLE 4. SUCCESSFUL P. FALCIPARUM SPOROGENY FROM TREATED AND UNTREATED CHILDREN AFTER FAILURE OF A SELF-ADMINISTERED PYRIMETHAMINE FIELD TRIAL IN GHANA

Subject's age (yrs)*	Pyrimethamine dosage (mg)	Before treatment			Day 3 after treatment		
		Number of <u>A. gambiae</u>			Number of <u>A. gambiae</u>		
		Fed	Exam.	Pos.	Fed	Exam.	Pos.
9	25.0	?	20	10	?	30	17
4	37.5	37	35	20	15	12	6
4	Nil	14	6	4	35	25	8
6	Nil	30	7	5	33	17	5

* Selected for P. falciparum gametocytes.