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Field Trials with Chlorproguanil in the Prophylaxis of Malaria in Ghana

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Chlorproguanil is one of the antimalarial drugs developed in recent years which have shown promise for field use in malaria eradication campaigns. It has been demonstrated to possess properties similar to those of proguanil but with a more persistent action. The author reports the results obtained in four field trials with this drug in Ghana.

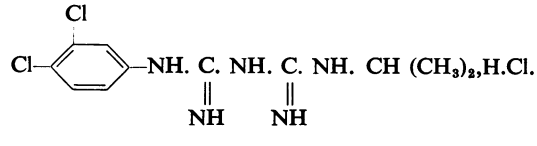
Chlorproguanil was shown to have a rapid action on asymptomatic parasitaemias of P. falciparum *and* P. malariae *(exclusive of crescents) in Ghanaian schoolchildren. In weekly doses of 20 mg it protected a group of children from reinfection for four weeks, but thereafter irregular dosage was associated with a small number of break-throughs by* P. falciparum. *By the end of six months there was strong evidence of* P. falciparum *resistance to the drug. Evidence was also found in* P. falciparum *infections of cross-resistance between chlorproguanil and pyrimethamine.*

Unlike pyrimethamine, chlorproguanil was not secreted in the maternal milk in sufficient quantity to be of therapeutic value to young, wholly breast-fed infants.

The author considers that, while this drug might serve as an effective weekly prophylactic in households where the regimen would be strictly followed, it would not be suitable for long-term community use in rural tropical Africa.

There can be little dissension among field malariologists from the views expressed by Bruce-Chwatt (1956) on the need for an antimalarial drug having all the characteristics necessary both for the clinician and for the epidemiologist. Until such a drug becomes available, however, each new product with proven high activity against human malaria must be subjected to comprehensive trial under all the rigour of field conditions in order to assess its potential contribution to the global eradication programme.

One such product, chlorproguanil (Lapudrine), was synthesized by I.C.I. research workers in the course of development work on the biguanides. Originally referred to as I.C.I. Compound 5943, and with a chemical formula of 3: 4 dichlorophenyl-isopropyl-diguanide hydrochloride,



chlorproguanil has been shown to possess properties similar to those of proguanil, but with a more persistent action. On the basis of results obtained in experimental exposures to *A. gambiae*-borne *Plasmodium falciparum* infection in Kenya, Robertson (1957) concluded that small weekly doses might be expected to produce complete causal prophylaxis against *falciparum* malaria. He also presented data indicating the occurrence of cross-resistance to chlorproguanil in a Malayan strain of *P. falciparum* resistant to proguanil.

This report sets out results obtained in four field trials with chlorproguanil in Ghana.

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FIELD TRIALS IN GHANA

TRIAL 1. EFFECT OF SINGLE-DOSE OF CHLORPROGUANIL ON ASYMPTOMATIC PARASITAEMIA

The object of this trial was to observe the speed of action of the drug on the asymptomatic parasitaemia encountered under local conditions of malarial endemicity.

Method

The youngest children attending primary school at the village of Boni in the Ashanti forest area were treated with a single 20-mg dose of the drug after examination for splenomegaly and collection of blood films. Their mean age was 6.9 ± 1.15 years; the spleen rate was 60.0%; and 37.1% showed liver enlargement below the right costal margin. Those found positive for trophozoites of malaria parasites were re-examined by thick blood film for three consecutive days (days 1-3) and for weeks 1, 2, 3 and 8 after the drug had been administered.

Results

Results obtained on 35 children thus observed are shown in Table 1. At the time of treatment (day 0) the asexual infections comprised 24 of *P. falciparum*, 4 of *P. malariae* and 7 mixed. Eight of the subtertian infections (including one associated with *P. malariae*) were cleared within 24 and all the others within 48 hours (day 2) after the single dose treatment. In contrast, *P. malariae* was observed to survive as late as week 1, but although these infections were certainly active on day 2, by day 3 only degenerating presegmenting forms were evident. This was particularly well marked in a child having an initial *P. malariae* density of 480/mm³ who, on day 3, showed a count of 120 presegmenters per mm³.

The treated group was kept free of asexual parasites for one week, but by week 3 *P. falciparum* had reappeared, and by week 8 37% of the children were reinfected. During the period of the observation, the drug showed no direct action on gametocytes of *P. falciparum*.

TABLE 1
EFFECT OF A SINGLE 20-mg DOSE OF CHLORPROGUANIL ON MALARIA PARASITAEMIA IN GHANAIAN SCHOOLCHILDREN

Time after treatment	No. examined	Positive trophozoites [a]						No. positive for *falciparum* gametocytes
		No.	PDI	F	FM	M	O	
Day 0	35	35	2.31	24	7	4	—	13
„ 1	35	28	2.11	17	6	5	—	13
„ 2	35	10	1.00	—	—	10	—	14
„ 3	35	8	1.00	—	—	8	—	16
Week 1	35	1	1.00	—	—	1	—	14
„ 2	35	0	—	—	—	—	—	13
„ 3	35	1	1.00	1	—	—	—	13
„ 8	30	13	2.46	10	1	1	1	2

[a] F = *P. falciparum*; M = *P. malariae*; O = *P. ovale*; PDI = Parasite density index.

TRIAL 2. PROPHYLACTIC VALUE OF WEEKLY DOSES OF CHLORPROGUANIL

The object of Trial 2 was to observe, over a period of six months, the degree of protection from reinfection—after initial clearance of parasites—conferred by weekly 20-mg doses of the drug to indigenous children under conditions of holoendemicity.

Method

This trial was carried out on 360 primary school children at five small villages spread out at intervals of approximately one mile along a good motor road in Ashanti, with Boni, the village used in Trial 1, at the centre. Because of the inevitable break during the school vacation, educational propaganda sessions were held with the village chiefs and elders, stressing the need for regular attendance by the children even during the holidays. To the teaching staff, the objects and methods of the trial were more fully outlined.

During 13-14 April 1959, immediately after the Easter school holidays, each child was examined for spleen and liver enlargement; blood films were taken, and a combined treatment of 300 mg base chloroquine plus 20 mg chlorproguanil was administered under direct supervision. Individual record cards were made, and by random allocation of these the children were assigned to two groups given the

TABLE 2

MALARIOMETRIC FINDINGS, BY AGE, IN TWO GROUPS OF GHANAIAN SCHOOLCHILDREN BEFORE TREATMENT WITH CHLORPROGUANIL

Group	Spleen			Liver		Blood		
	No. examined	Enlarged		Enlarged		No. examined	Positive	
		No.	%	No.	%		No.	%
5-9 years:								
Treated	145	85	58.6	40	27.6	132	89	67.4
Control	108	59	54.6	32	29.6	98	62	63.2
Total	253	144	56.9	72	28.5	230	151	65.6
10-14 years:								
Treated	47	24	51.1	4	8.5	47	35	74.5
Control	60	23	38.3	5	8.3	60	32	53.3
Total	107	47	43.9	9	8.4	107	67	62.6

following weekly dosages beginning seven days after the above preliminary treatment:

Group 1. Treated: 20 mg chlorproguanil

Group 2. Control: 50 mg ascorbic acid

The tablets were given during roll call on Monday mornings only, and no effort was made to seek out absentees. One corner of the cards for Group 1 was cut away to simulate the partially " cut " or scored chlorproguanil tablets, and thus, by association, to simplify the differentiation of the two groups and their appropriate medicament.

Weekly administration and attendance registration were made during term by the class teachers with occasional assistance by Malaria Unit staff. During the school holidays (2½ weeks in August) the children met the latter by arrangement at the school buildings for treatment. Periodic random blood survey samples were collected, and on 22 October 1959, at the end of the six-month period, all children were re-examined as before.

Anopheles mosquitos collected during morning house-spray catches were dissected in April and August for sporozoites.

Results

Although 360 children were examined on the first day of the trial (week 0), blood film results are available for only 337 (Table 2). Spleen and parasite rates of 56.9% and 65.6% respectively were recorded among the age-group 5-9 years; and of 43.9% and 62.6% respectively among the older group aged 10-14 years. The average enlarged spleen for both groups was 1.71. Of the younger group 28% and of the older group only 8% showed hepatic enlargement below the right costal margin. Parasite findings conformed with the general picture for West Africa. Thus in the younger group, with a parasite formula of F 66.1, M 32.2, O 1.7,[1] the density index was 1.85 and the *P. falciparum* gametocyte rate 22.6%. Among the group 10-14 years the corresponding results were F 70.0, M 26.2, O 3.8, with a density index of 1.40 and a crescent rate of 19.6%.

TABLE 3

ATTENDANCE RECORDS OF TWO GROUPS OF SCHOOLCHILDREN PARTICIPATING FOR 6 MONTHS IN A WEEKLY DRUG TRIAL IN GHANA

No. of treatments	Attendance			
	Treated		Control	
	No.	%	No.	%
26	37	21.6	25	17.0
25-23	89	52.1	77	52.4
22-20	31	18.1	37	25.2
19-17	10	5.9	7	4.7
16-14	4	2.3	1	0.7
Total	171	100.0	147	100.0

During the course of the trial no general symptoms of toxicity were reported. At the beginning, the children grimaced at the bitter taste of the chlorproguanil tablet, but its small size was conducive to easy swallowing with water.

A total of 42 (18 treated and 24 control) children failed to complete the trial, partly because their families had moved away from the area, but partly also because at one school the number in the youngest class had obviously been temporarily augmented on the first day by children who were not normally enrolled but whose parents had pressed the teachers for their inclusion. The attendance record of the 318 who completed the course is shown in Table 3. (In Table 3, $\chi^2=4.31$; n=4; and $0.50 < P > 0.30$.) Over 90% of each group observed

[1] F = *falciparum*; M = *malariae*; O = *ovale*.

TABLE 4

PERIODIC MALARIA PARASITE RATES AFTER INITIAL CLEARANCE OF PARASITAEMIA IN TWO GROUPS OF CHILDREN DURING A TRIAL WITH WEEKLY DOSES OF 20 mg CHLORPROGUANIL

Group	Treatment week																	
	Week 0			Week 4			Week 10			Week 16			Week 22			Week 26		
	Examined	Pos.	%	Examined	Pos.	%	Examined	Pos.	%	Examined	Pos.	%	Examined	Pos.	%	Examined	Pos.	%
Treated	179	124	69.3	45	2[a]	4.4	111	6	5.4	83	4	4.8	147	9	6.1	170	10	5.9
Control	158	94	59.5	55	3[b]	5.4	83	43	51.8	133	73	54.9	116	72	62.1	145	91	62.7

[a] Both with *falciparum* gametocytes only.
[b] One with *falciparum* gametocytes only.

(91.8% treated and 94.5% control) were present for 20 or more of the 26 weekly treatments, and this pattern was repeated at all five schools. Although, therefore, it was not possible to determine exactly the cause of absenteeism, deliberate avoidance of the unpalatable chlorproguanil tablets did not appear to be an important factor.

The results of periodic blood film examinations are shown in Table 4, but since the parasite rates for the age-groups 5-9 and 10-14 years were virtually identical at each examination, they have been combined for purposes of simpler presentation. As may have been expected from Trial 1, few of the children, even in the control group, had asexual parasitaemia by week 4 after the initial treatment. Thus, although parasite rates of 4.4% and 5.4% were recorded for treated and control groups respectively, both of the positives in the former and one of three in the latter were gametocytes of *P. falciparum* carried over from week 0. By week 10, however, new *P. falciparum* infections began to appear in small numbers among the treated and in moderately high proportion among the controls. On week 26 when the trial ended, the parasite rate in the treated group was still low at 5.9%, while in the control group the infection rate (62.7%) had reverted to the pre-treatment levels of week 0.

The 37 children who received the full 26-week course of chlorproguanil remained free from infection whenever they were included in the several survey samples. Positive findings among the others were all scanty *P. falciparum* trophozoites without gametocytes. Although some of the reinfections among the treated group could be related to absences from tablet parade, that was not always the case. Thus the 10 children of this group found positive on week 26 had been marked present at tablet distribution for unbroken periods ranging from one to 18 weeks prior to that examination. Although no treated child was positive on any two successive examinations, these break-throughs suggested that a certain degree of selection of resistant forms of *P. falciparum* was in progress, but opportunity did not arise to check this by direct observation in the field.

Reinfections among the control group were predominantly *P. falciparum*, with gametocytes reappearing by week 10, but *P. ovale* was encountered on week 16 and at the two later blood film surveys. *P. malariae* (mixed with *P. falciparum*) was found once only, on week 26.

There was a reduction in the spleen rate among the treated groups, significant at the 0.1% level, from 58.6% on week 0 to 19.4% of 129 examined on week 26 in the 5-9-year group; and from 51.1% to 14.6% of 41 examined in the older group. In the control children spleen rates at 57.7% and 43.6% respectively for these age-groups remained statistically unchanged from those shown for week 0 in Table 2. Liver enlargement on week 26 remained at pre-treatment levels for both treated and control groups.

An indication of the level of malaria transmission at the villages during the time of the trial is shown by the results of gland dissection of house-caught *A. gambiae* (Table 5), with daily inoculation rates of 0.02 and 0.01 at the start and during the middle of the trial period.

TRIAL 3. SECRETION OF CHLORPROGUANIL IN MATERNAL MILK

There is a growing interest in medication of salt as a method of distributing anti-malarial drugs on a community scale. Since chloroquine as employed in

TABLE 5
RESULTS OF SPRAY CATCHES AND DISSECTION OF HOUSE-RESTING *ANOPHELES* IN VILLAGES OF THE CHLORPROGUANIL TRIAL AREA

Date	No. of rooms	*A. gambiae*		
		No. per man	No. examined	Positive gland dissections
April	12	1.35	27	1
August	18	0.35	16	1

Brazil (Pinotti & Soares, 1956) is not secreted in the maternal milk (Soares et al., 1957) there may be need, in tropical Africa, to incorporate a drug which by this route would reach the infant who is usually weaned late in his second year. Clyde, Shute & Press (1956) have shown that pyrimethamine fulfils this requirement, but the increasing records of *P. falciparum* resistance after mass use of that drug in this region justified investigation of chlorproguanil in this respect.

Method

At two neighbouring villages on the main highway in the newly designated Volta Region of Ghana (formerly Togoland) nursing mothers attending community development classes in home craft participated in the trial. Each received and swallowed a single treatment of chlorproguanil, pyrimethamine or placebo (ascorbic acid) on day 0, and thick blood films were collected from their infants aged 3-24 months. Fifty-three of those found positive for malaria trophozoites were re-examined on the seventh day after treatment.

Results

The results obtained (Table 6) showed that whereas parasitaemia of moderately high density was cleared in 10 of 18 infants after maternal treatment with pyrimethamine, only one of 22 in the chlorproguanil group was so cleared—a difference which was highly significant ($\chi^2=20.9$; n=4; P=0.0037). These preliminary results therefore suggested that chlorproguanil was not transferred in sufficient quantity *via* the maternal milk for practical purposes of suppressing malaria infections in nursing infants. Even for pyrimethamine the data from this small trial indicated greatly reduced efficacy in infants aged over 12 months, and although these older infants were invariably described as being only partly breast-fed, their supplementary feeds were said to be of the porridge and pap variety, rather than portions from the household meal. Since the age composition of the groups under consideration in this trial was comparable, however, the results are not influenced by this additional observation on pyrimethamine.

TABLE 6
BLOOD FILM RESULTS FOR MALARIA TROPHOZOITES IN INFANTS BEFORE (DAY 0) AND AFTER (DAY 7) SINGLE-DOSE TREATMENT OF THEIR NURSING MOTHERS

Single-dose maternal treatment	Infant blood films				
	Day 0		Day 7		
			Number		
	No. pos.	Parasite density index	Examined	Pos.	Parasite density index
Pyrimethamine					
25 mg	8	5.00	8	3	4.33
50 mg	10	6.20	10	5	3.80
Chlorproguanil					
20 mg	10	5.70	10	10	5.40
40 mg	12	5.01	12	11	5.91
Ascorbic acid					
50 mg	13	3.92	13	13	4.08

TRIAL 4. CROSS-RESISTANCE WITH PYRIMETHAMINE

Before the results for week 26 in Trial 2 were available, with their suggestion of development of resistance to chlorproguanil in *P. falciparum*, an opportunity arose to investigate and confirm *P. falciparum* resistance to pyrimethamine in a village in Ghana [1] where chlorproguanil had not hitherto been used.

Nine children who remained positive for *P. falciparum* trophozoites eight days after a supervised 25-mg dose of pyrimethamine received 20 mg of chlorproguanil. On day 3 after this latter treatment all but one were still positive and on day 7 six continued to show asexual forms, though at greatly reduced density. In view of the results shown in Table 1 these findings indicated the occurrence of cross-resistance between pyrimethamine and chlorproguanil. This was not surprising since Robertson, Davey & Fairley (1952) had already recorded cross-resistance between pyrimethamine and proguanil.

[1] Charles, L. J. (1960) *Aftermath of a field trial in self-administered pyrimethamine in a Ghanaian community: The appearance of* P. falciparum *resistance* (unpublished working document WHO/Mal/260).

DISCUSSION

From the results of Trial 1 it would appear that there was higher susceptibility to single-dose chlorproguanil in the *P. falciparum* of forest areas of central Ghana than in that of Northern Nigeria. Archibald & Robertson (1959) observed three of 25 such infections surviving 48 hours after swallowing a 20-mg tablet, and one of these persisted beyond four days, although it was cleared within 48 hours of a repeat dose of the same drug. The more rapid action recorded here among asymptomatic schoolchildren tended to be confirmed by a small number of observations on overt *falciparum* malaria in infants at the Ho hospital, where high parasite densities were generally cleared within 48 hours of single-dose chlorproguanil. Of greater import, however, was the protection of 35 children from *P. falciparum* infection for one clear week (Table 1) after a 20-mg dose of the drug. This appeared to confirm Robertson's (1957) conclusions on the prophylactic potential of chlorproguanil against that species. His demonstration of its inhibitory effect on the development of sporozoites in Kenya rendered unimportant its failure to reduce directly the number of gametocyte carriers as recorded in the present trials.

For *P. malariae*, attention has already been directed to the persistence of undeveloping presegmenters even after single-dose treatment with the 4-aminoquinolines (Charles, 1958), followed by their disappearance without additional medication. Although this was not considered as *de facto* evidence of lower susceptibility of quartan parasites to the drugs then under consideration, the present results with chlorproguanil, particularly in the mixed infections, clearly illustrate the greater tolerance of *P. malariae* to this synthetic drug, as recorded for others by Young & Eyles (1948) and Schneider & Méchali (1948).

The preliminary dose of chloroquine plus chlorproguanil administered in Trial 2 was considered adequate to clear the children of all asexual malaria parasites and pre-erythrocytic forms of *P. falciparum*. Under ideal conditions of distribution, therefore, the proven causal prophylactic properties of weekly chlorproguanil might have been expected to provide complete protection of the treated group from *P. falciparum* infection and erythrocytic forms of the other two species encountered in the area. This estimate was actually realized and maintained—at least until week 4 (Table 4)—but by week 10 *P. falciparum* had began to break through the chemoprophylactic defence of those children who attended irregularly on tablet day. Because of the ensuing process of partial exposures to the drug, selection of resistant forms of the parasite had occurred by week 26.

Since this result developed at school, where, without coercion and in the absence of any obvious inherent deterrent, regular attendance is a normal expectation, the outcome of a comparable trial with the drug in a composite village community can be safely predicted; for the cumulative experience of malariologists in tropical Africa suggests that absentee rates of 8%-16% are usual in field programmes involving personal tablet administration over a period of time. This forecast of the likelihood of chlorproguanil resistance developing in a village community was enhanced by the results of Trial 4, which showed that resistance to pyrimethamine, developed during irregular administration of that drug, confers a cross-resistance in *P. falciparum* to chlorproguanil.

In the absence of mass use of the biguanides or diaminopyrimidines, chlorproguanil would serve as an effective weekly prophylactic in responsible and conscientious households, but it can hardly be considered suitable, *per se*, for long-term community use in rural tropical Africa.

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RÉSUMÉ

Tant que le médicament antipaludique idéal n'est pas découvert, les substances chimiothérapiques dont les qualités sont reconnues doivent être soumises à des essais pratiques strictement contrôlés, afin que soient évalués les services qu'elles peuvent rendre dans les campagnes d'éradication.

Le chlorproguanil, dont la persistance dans l'organisme est supérieure à celle du proguanil, a été récemment proposé. Essayé au Kenya avec succès, il vient de faire l'objet d'une étude au Ghana. L'auteur expose les résultats relatifs à la vitesse d'action de cette substance dans les cas de parasitémie asymptomatique, dans certaines conditions d'endémicité. Le chlorproguanil a été administré à des enfants d'âge scolaire, à raison de doses hebdomadaires de 20 mg. L'action sur la parasitémie asymptomatique à *P. falciparum* et *P. malariae* (sauf les formes en croissants) a été rapide. Les enfants ont été protégés des réinfections pendant 4 semaines, mais, à la suite d'une administration moins régulière, des rechutes à *P. falciparum* ont été observées. Après 6 mois, il s'avéra que *P. falciparum* était résistant à ce médicament, et que cette résistance s'étendait à la pyriméthamine.

D'autre part, l'intérêt évident que présentent certains antipaludiques de passer dans le lait maternel, et de protéger ainsi les très jeunes enfants nourris au sein jusqu'à la fin de leur deuxième année, a motivé l'administration de proguanil à de jeunes mères. Mais les résultats montrèrent que, contrairement à la pyriméthamine, le chlorproguanil ne passe pas dans le lait en quantité suffisante pour protéger du paludisme les très jeunes enfants.

Etant donné la proportion (8-16 %) de sujets d'une communauté qui ne prennent pas régulièrement le médicament qu'ils sont censés absorber, il ne paraît pas que le chlorproguanil puisse être recommandé pour une campagne de longue durée en Afrique tropicale rurale, mais il peut avoir une action prophylactique utile dans les foyers où la dose hebdomadaire prescrite est consciencieusement et régulièrement absorbée.

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