SHORT REPORT: EFFECTS OF PYRONARIDINE ON GAMETOCYTES IN PATIENTS WITH ACUTE UNCOMPLICATED FALCIPARUM MALARIA

PASCAL RINGWALD, FLEURETTE SOLANGE MECHE, AND LEONARDO K. BASCO

Institut de Recherche pour le Développement (ORSTOM) and Laboratoire de Recherche sur le Paludisme, Laboratoire Associé Francophone 302, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroon

Abstract. The effects of pyronaridine and chloroquine on mature *Plasmodium falciparum* gametocytes were compared in 161 patients treated with chloroquine or pyronaridine. Neither pyronaridine nor chloroquine showed gametocytocidal activity. The relative risks of post-treatment gametocytemia after pyronaridine and chloroquine treatment in the presence of chloroquine-resistant isolates were 1.25 and 11.5, respectively, suggesting that the use of chloroquine was associated with a high risk of favoring post-therapeutic gametocytemia in chloroquine-resistant infections.

The intense and continuous transmission of *Plasmodium falciparum* is one of the important factors that maintain the high prevalence of malaria in most of tropical Africa. The available measures to reduce malaria transmission include vector control and the use of gametocytocidal drugs. Vector control in Africa has not had much impact on malaria control, except in pilot programs.[1] The currently available first- or second-line (chloroquine, amodiaquine, sulfadoxine-pyrimethamine) and third-line (quinine) drugs in Africa do not exhibit any direct gametocytocidal action on *P. falciparum*.[2] Pyronaridine is a new synthetic drug that is undergoing preclinical and clinical evaluation and may replace chloroquine in Africa.[3,4] We studied and compared the effects of pyronaridine and chloroquine on gametocytes in patients with acute uncomplicated falciparum malaria.

The study was part of the clinical trials involving 184 symptomatic, malaria-infected African patients (96 adults and 88 children between 5 and 15 years of age) in Yaoundé, Cameroon.[4,5] Patients were treated with either 25 mg/kg of chloroquine phosphate (n = 93; 10 mg/kg on days 0 and 1 and 5 mg/kg on day 2) or 32 mg/kg of pyronaridine tetraphosphate (n = 91; 16 mg/kg on day 0 in two divided doses and 8 mg/kg on days 1 and 2) after informed consent was obtained from the patients or their guardians. The study was approved by the Cameroonian National Ethics Committee. Giemsa-stained thick blood smear was examined for the detection and quantification of gametocytes. The clinical conditions and asexual parasitemia were monitored on days 0, 1, 2, 3, 4, 7, and 14 on an out-patient basis. Because of the slower developmental rate of gametocytes (8–10 days), compared with the 48-hr cycle of *P. falciparum* asexual erythrocytic forms,[6] gametocyte count was monitored on days 0, 3, 7, and 14. Gametocytes were counted against 3,000 white blood cells, and gametocyte density was determined from the white blood cell count and expressed as the number of gametocytes/μl of blood.

Thick blood smears were considered negative if no gametocyte was seen after counting 3,000 white blood cells. The patients were assigned to one of the following groups for the analysis of parasitologic responses of gametocytes: 1) absence of gametocytes during the 14-day follow-up period, including day 0, 2) pretreatment presence of gametocytes (which allows the evaluation of a possible gametocytocidal effect of antimalarial drugs), and 3) post-treatment appearance of gametocytes, defined as the absence of ga-

metocytes on day 0 and appearance of gametocytes on days 3, 7, and/or 14. *In vitro* drug sensitivity assay was performed to determine whether the patients were infected with chloroquine-sensitive or chloroquine-resistant isolates.[7] The threshold 50% inhibitory concentration (IC_{50}) value for chloroquine resistance was fixed at > 100 nM.

Data were presented on 2×2 contingency tables, and proportions were compared by Fisher's exact test. The relative risks of patients with no detectable gametocytes on day 0 who developed gametocytemia after treatment were determined by treatment group and *in vitro* chloroquine resistance. Since the patients were classified by three criteria (treatment, drug effect on gametocytes, and chloroquine resistance), the Mantel-Haenszel chi-square test was used to test whether there is any confounding effect of the contingency tables that were combined. If the relative risk calculated from the Mantel-Haenszel chi-square test differed by < 15 % from the relative risk calculated from pooled contingency tables, an association between the first two criteria and the third criterion was excluded. Quantitative variables were compared using the *t*-test.

Of the 184 patients enrolled in the study, 161 (81 in the pyronaridine group and 80 in the chloroquine group) completed the 14-day follow-up. Eighty-seven of 161 patients (54 %) had no detectable gametocytes in any of their initial or follow-up smears (Table 1). Eight (9.9 %) of 81 pyronaridine-treated patients and three (3.8 %) of 80 chloroquine-treated patients had *P. falciparum* gametocytes in the peripheral blood before treatment. The mean pretreatment gametocytemia (range) was 85 gametocytes/μl (12–510 gametocytes/μl) in these patients. In the pyronaridine-treated group, two children with initial gametocytemias of 12 and 42 gametocytes/μl had no gametocytes on day 14. In two other children, the initial gametocytemia (12 and 510 gametocytes/μl) increased 3–13-fold on either day 3 or 7, then decreased to approximately the pretreatment level on day 14. In one adult patient, the initial gametocyte level (84 gametocytes/μl) increased 16-fold on day 3 and remained at a level that was 13–20-fold higher than the pretreatment level until day 14. In three patients (one adult and two children), the level of gametocytemia remained relatively constant (less than a three-fold fluctuation) during the entire follow-up period. In the chloroquine-treated group, two children with initial gametocytemias of 18 and 48 gametocytes/μl had a relatively narrow fluctuation of gametocytemia (less than three-

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TABLE 1

Effect of pyronaridine and chloroquine on *Plasmodium falciparum* gametocytes in symptomatic patients*

Classification	Number of patients	
	Children	Adults
Absence of gametocytes		
Pyronaridine	9	17
Chloroquine	31	30
Pretreatment presence of gametocytes (on day 0)		
Pyronaridine	6	2
Chloroquine	2	1
Post-treatment appearance of gametocytes (on days 3, 7, and/or 14)		
Pyronaridine	26	21
Chloroquine	6	10

* The comparison of proportions in the three 2 × 2 contingency tables by Fisher's exact test showed no statistical difference (*P* > 0.05).

TABLE 2

Post-therapeutic gametocytemia following pyronaridine and chloroquine treatment

Characteristics	Pyronaridine	Chloroquine
Number of patients (children/adults)	47 (26/21)	16 (6/10)
Chloroquine-resistant parasites*	26/45	14/15
Gametocyte count†		
Day 3		
Number with positive gametocytes	6	4
Mean (range) (gametocytes/µl)	14 (6–78)	25 (12–78)
Day 7		
Number with positive gametocytes	44	16
Mean (range) (gametocytes/µl)	49 (6–372)	40 (12–120)
Day 14		
Number with positive gametocytes	34	12
Mean (range) (gametocytes/µl)	35 (6–720)	40 (6–1,380)

* Results of *in vitro* assay were interpretable in 45 of 47 isolates from pyronaridine-treated patients and 15 of 16 isolates from chloroquine-treated patients.

† Gametocyte count is expressed as geometric mean gametocytes/µl of blood. The arithmetic mean counts on days 3, 7, and 14 compared between the two treatment groups showed no statistically significant difference by the *t*-test (*P* > 0.05).

fold) until day 14. Both of these children cleared their asexual parasitemia before day 7 and the parasites were chloroquine-sensitive. One chloroquine-treated adult presented a 10–16-fold increase in gametocytes on days 3 and 7 and a gametocyte level similar to the pretreatment level on day 14. This patient failed to clear the asexual parasitemia and harbored a chloroquine-resistant isolate.

Compared with the gametocytocidal efficacy of primaquine (gametocyte clearance within 4–8 days),[8] our data based on a small number of gametocytemic patients suggest the absence of gametocytocidal effect of pyronaridine and chloroquine against the gametocytes of *P. falciparum*. Our data on the absence of gametocytocidal effect of chloroquine on mature *P. falciparum* gametocytes are in agreement with those of previous studies.[2,9] However, our observation that chloroquine-treated patients infected with chloroquine-sensitive parasites had lower gametocytemia, compared with the patient with chloroquine-resistant parasites, is consistent with the fact that chloroquine exerts an inhibitory action against immature gametocytes.[2]

Forty-seven (64%) of 73 patients in the pyronaridine group and 16 (21%) of 77 patients in the chloroquine group without detectable gametocytes on day 0 subsequently developed gametocytemia. The evolution of gametocytemia in these patients is summarized in Table 2. Although the mean gametocyte count did not differ significantly in patients with positive gametocyte counts (*P* > 0.05, by *t*-test), the difference in the number of gametocyte-positive patients in the treatment groups was evident on days 7 and 14. The absence of post-treatment gametocytemia in the majority of chloroquine-treated patients is in agreement with the results of a previous study.[10] The underlying reason may be related to the activity of chloroquine against immature gametocytes, which are sequestered in deep organs during the maturation process. Subjects treated with pyronaridine were more likely to have post-treatment gametocytemia than subjects treated with chloroquine (relative risk = 3.26, 95% confidence interval [CI] = 2.16–4.91; *P* < 0.001), suggesting that pyronaridine has either no activity or less activity than chloroquine against immature gametocytes.

The relative risks of post-treatment gametocytemia after pyronaridine and chloroquine treatment in the presence of chloroquine-resistant isolates were 1.25 (95% CI = 0.88–

1.77) and 11.5 (95% CI = 1.60–82.7), respectively. Among the pyronaridine-treated patients in whom gametocytes were detected during the post-treatment period, 26 (58 %) of 45 were infected with chloroquine-resistant isolates, which corresponds to the expected proportion (50–60%) of the chloroquine-resistant isolates in Yaoundé.[7] Fourteen of 15 isolates obtained from chloroquine-treated patients with post-therapeutic appearance of gametocytes were chloroquine-resistant. This observation agrees with other studies that have suggested that chloroquine therapy in a chloroquine-resistant endemic region may favor the survival and selection of gametocytes carrying the resistant gene.[11-13] These preliminary results, which suggest that patients who are infected with chloroquine-resistant isolates are more likely to become gametocyte carriers than those who are infected with chloroquine-sensitive parasites, need to be evaluated in different patient populations under various epidemiologic conditions. Further studies are needed to establish whether post-therapeutic gametocytes are infective to mosquitoes, leading to an increased transmission of chloroquine-resistant *P. falciparum* strains.

Authors' address: Pascal Ringwald, Fleurette Solange Meche, and Leonardo K. Basco, Institut de Recherche pour le Développement (ORSTOM) and Laboratoire de Recherche sur le Paludisme, Laboratoire Associé Francophone 302, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), BP 288, Yaoundé, Cameroon.

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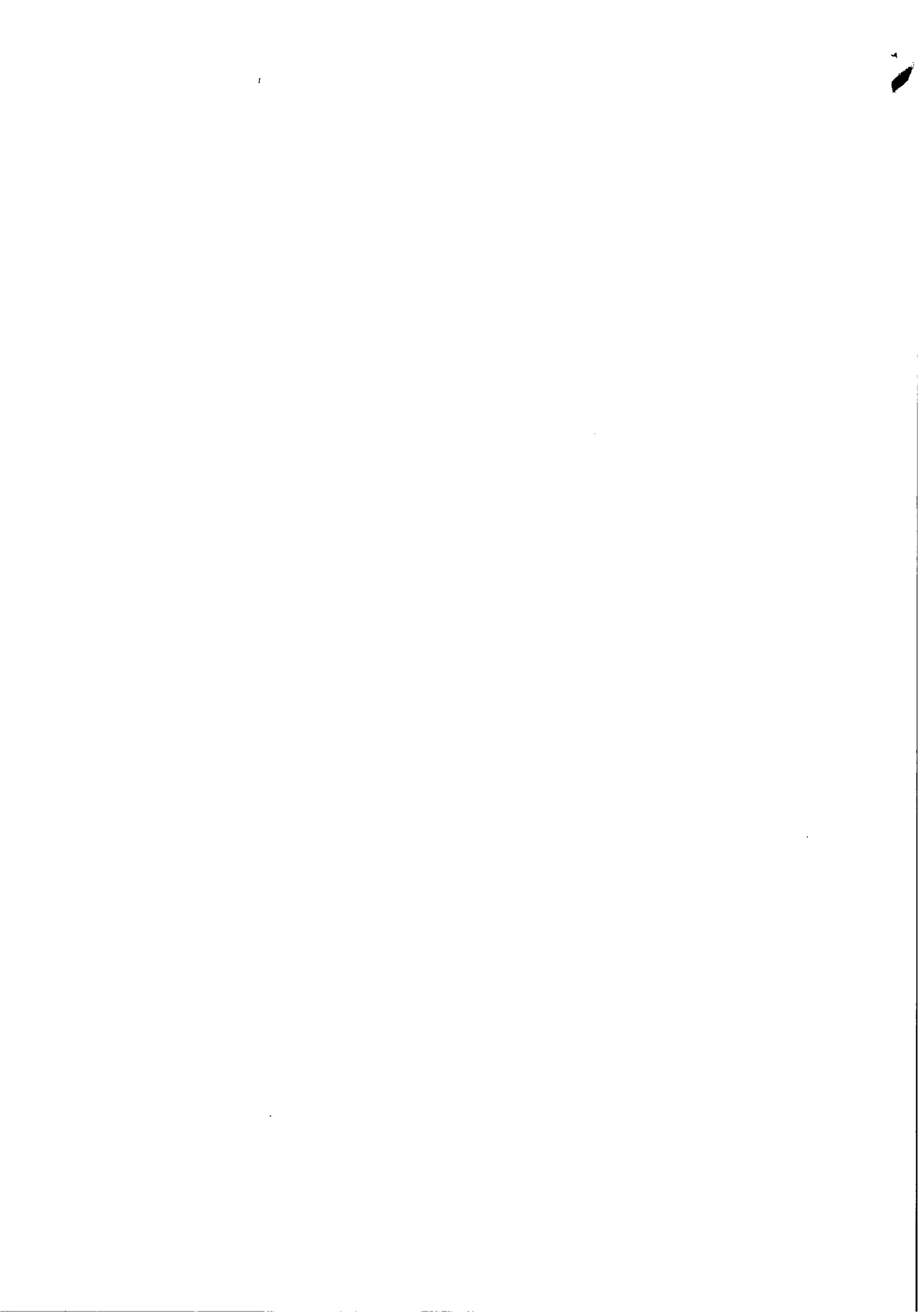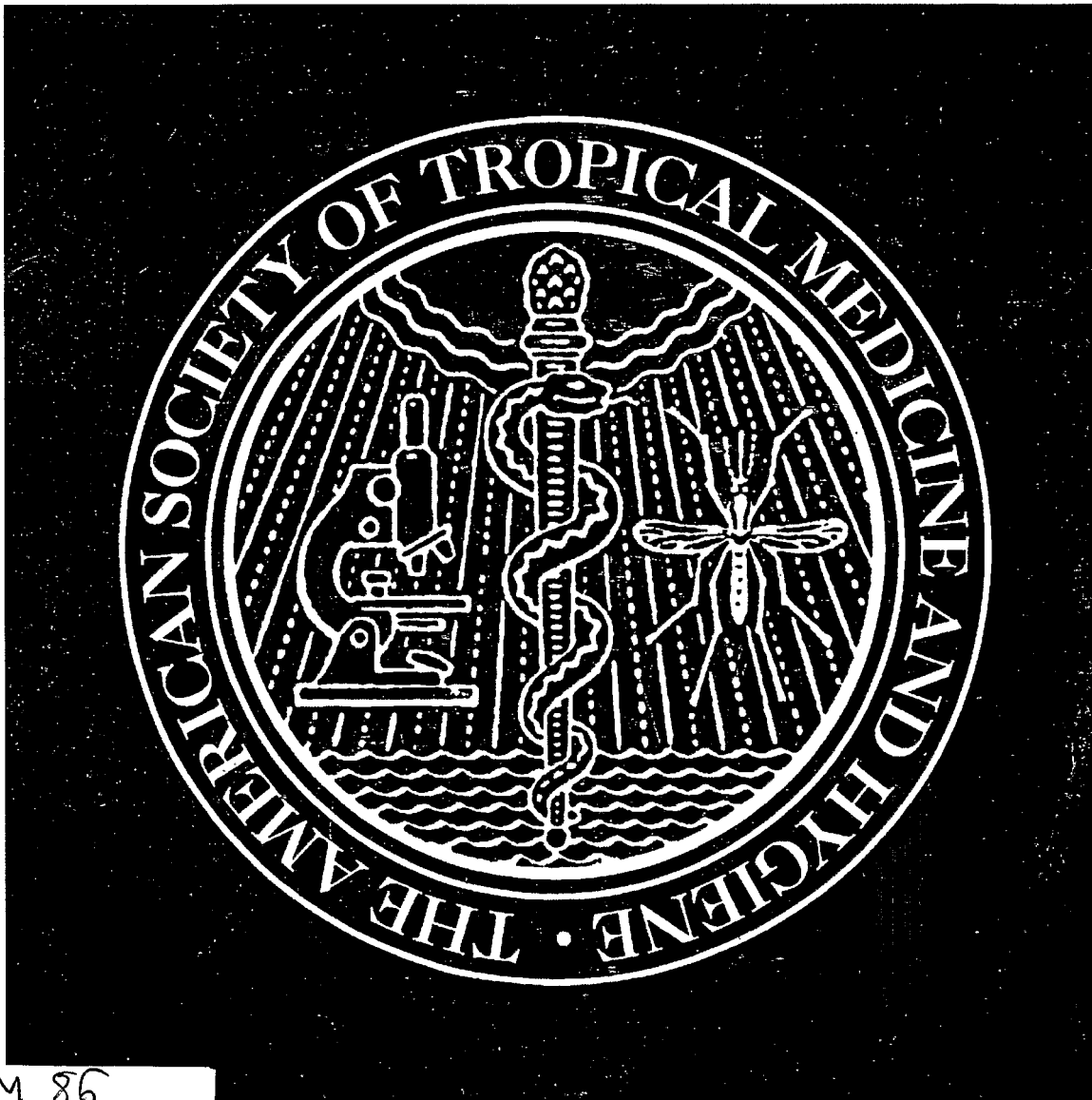
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