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PYRIMETHAMINE RESISTANCE IN *PLASMODIUM VIVAX* MALARIA

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SYNOPSIS

P. vivax infection (Korean, St Elizabeth or Chesson strain) was induced in 17 neurosyphilitic patients. Pyrimethamine in single doses of either 25, 50, 100 or 200 mg was given to test the schizontocidal and sporontocidal effects.

The first single-dose treatment of 25 mg or 100 mg was given between the 8th and 61st days of parasite patency and gave moderately rapid schizontocidal and very rapid sporontocidal effects. All observed cases relapsed.

The second treatment, usually three weeks or longer after the first and with the same or higher doses, had either a diminished effect or none on the schizogonous and sporogonous cycles. Subsequent treatment, even at weekly intervals, had no effect.

The resistant quality was undiminished in subsequent infections transmitted by mosquito bites, by the injection of preserved sporozoites, or by transfusion of infected blood. Preserving sporozoites or erythrocytic parasites at very low temperatures did not materially affect the resistant quality.

In view of the evidence presented, it appears that resistance could also occur in the field when large single doses of pyrimethamine alone are given at less than monthly intervals to febrile persons having active *P. vivax* infections.

Pyrimethamine, one of the most recently developed antimalarial drugs, has been tried in several regimens against induced infections of *Plasmodium vivax*. Early in the tests it was observed that a relapsing infection responded less well to the second than to the initial treatment. As this apparently was related to the development of resistance to the drug, experiments were designed to determine some of the factors concerned with the occurrence, persistence, and transmissibility of resistance. The results are given in this report.

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Materials and Methods

The St Elizabeth, Korean, and Chesson strains of *P. vivax* were used. The infections were induced in white neurosyphilitic patients by the injection of either fresh blood or blood that had been preserved by maintenance at about −78° C, or by sporozoites. The latter were transmitted by the bites of infected mosquitos or by the injection of a sporozoite suspension which had been preserved at low temperature (about −78° C) by the method of Jeffery & Rendtorff (1955).

The pyrimethamine was given as single doses of 25, 50, 100, or 200 mg, except in one case where 50 mg were given on each of two days. The first doses were given between the 8th and 61st days of parasite patency, but usually after the second week of patency. The 100-mg and 200-mg doses were used against the Chesson strain and the lower doses against the St Elizabeth and Korean strains. Doses subsequent to the first were given either when the infection had not been cleared from the blood-stream or when the infection relapsed. Parasite densities were determined by the Earle-Perez technique.

To determine the sporontocidal effect of the drug, mosquitos were applied before and usually daily for several days after the drug administration. In certain cases, feedings were done at 2-hour and 4-hour intervals for 24 hours after drug administration.

The mosquitos used were *Anopheles quadrimaculatus* (Q_1 strain) and *A. freeborni* (F_1 strain). After the infective feedings they were maintained at 76° F ± 2° F (24.5° C ± 1° C) and at a high relative humidity for 16-18 days. Usually 20 mosquitos were dissected to determine the presence of infection. On the 8th to 10th day of incubation 10 mosquitos were dissected to determine the density of oocysts; if the infection was light or absent, an additional 10 were dissected. After about two weeks' incubation 10 more mosquitos were dissected to determine the presence of sporozoites. The other details in handling the infected mosquitos have been described elsewhere (Burgess & Young, 1944).

When the term " infected mosquitos " is used in this report it means the presence of sporozoites or healthy oocysts capable of producing sporozoites, unless otherwise noted.

Observations

Seventeen patients with *P. vivax* were given pyrimethamine one or more times (Table 1). In three cases (patients 1252, 1256, and 1307) the linear antecedents of the parasites had been in contact with the drug (Fig. 1); these will be discussed later.

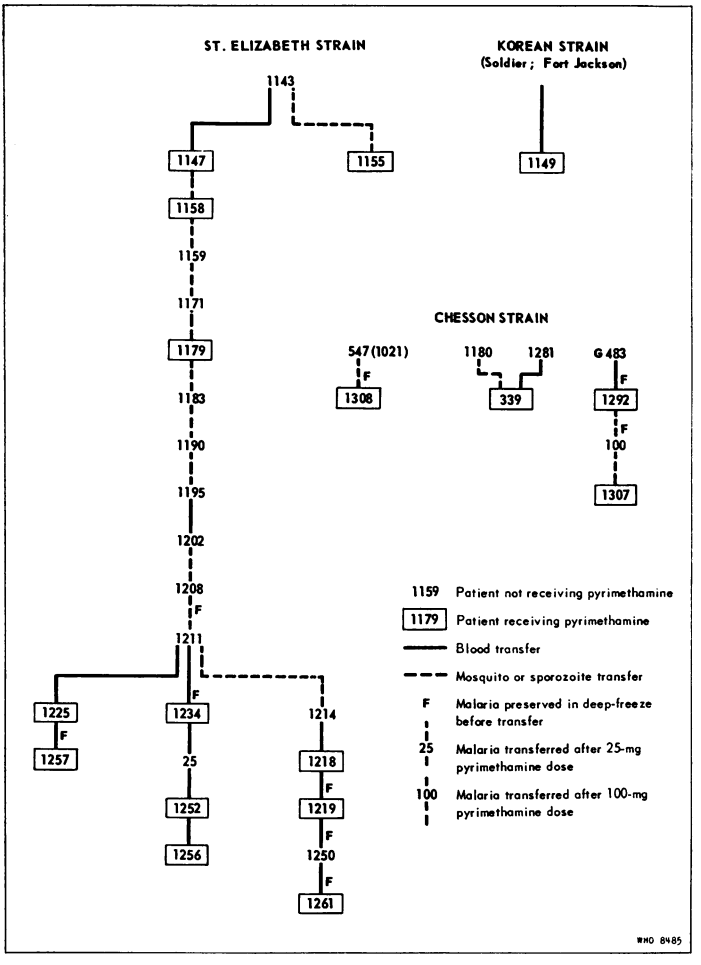
In 14 cases representing the first contact with the drug, the parasites were removed from the blood-stream in from four to eight days (median,

TABLE 1. RESPONSE OF *P. VIVAX* TO PYRIMETHAMINE, SHOWING THE EFFECT ON THE PARASITAEMIA AND INFECTIVITY AT THE FIRST AND SUBSEQUENT TREATMENTS

Patients	Contact with drug [a]	Quantity of drug (mg)	Average number of parasites per ml	Percentage of parasites remaining			Days to clear		Number relapsing/ total followed	Days to relapse	Mosquito infectivity after treatment	Remarks
				days after treatment			range [b]	median				
				1	2	8						
Group A [c]	1	25	6 863	59	9	0	4-8	6	6/6	19-58	nil	10 St Elizabeth strain, 1 Korean strain (1149)
339, 1292, 1308 [d]	1	100	12 516	60	12	0	6-8	8	3/3	16-23	nil	All Chesson strain
1234	2	50	50	20	100	600	43		1/1	65		Group A relapse. St Elizabeth strain
1252	2	25	1 170	88	53	41	c.p.					Subinoculated from 1234 before second treatment. St Elizabeth strain
1256	2	25	10 711	94	26	25	c.p.					Subinoculated from 1252 before treatment of 1252. St Elizabeth strain
1252	3	50	480	106	33	119	62		1/1	83		St Elizabeth strain
1292, 1308 [d]	2	100	25 199	88	17	21	c.p.				reduced normal	Relapses. Chesson strain
1292	3	100	1 521	68	59	128	c.p.				normal	Chesson strain
1307 [d]	4	100	3 060	58	52	252 [e]	c.p.				normal	Subinoculated from 1292 after third treatment. Chesson strain
1307 [d]	5	100	7 712	88	56	79 [e]	c.p.				normal	Chesson strain
1307 [d]	6	200	5 580	110	5	[f]					normal	Chesson strain

[a] Number of times the particular line of parasites had been treated
[b] c.p. = continuous parasitaemia until re-treated
[c] This group comprised 13 patients (1147, 1149, 1155, 1158, 1179, 1218, 1219, 1225, 1234, 1257, 1261, 1252, 1256), 3 of them sporozoite-inoculated.
[d] Sporozoite-inoculated
[e] 7th day after treatment
[f] Treated with 600 mg amodiaquine on day 3

FIG. 1. LINEAR PASSAGES OF *PLASMODIUM VIVAX*



seven days) following either the 25-mg or 100-mg dose. All cases (nine) which were followed long enough relapsed (median, 22 days). Eight of these were blood-induced infections and one was sporozoite-induced.

Upon relapsing the infection in patient 1234 was subinoculated to patient 1252. Patient 1234 then was given 50-mg of pyrimethamine, which was twice the amount of the initial dose. The parasites rapidly increased temporarily, disappeared on day 43, and reappeared on day 65.

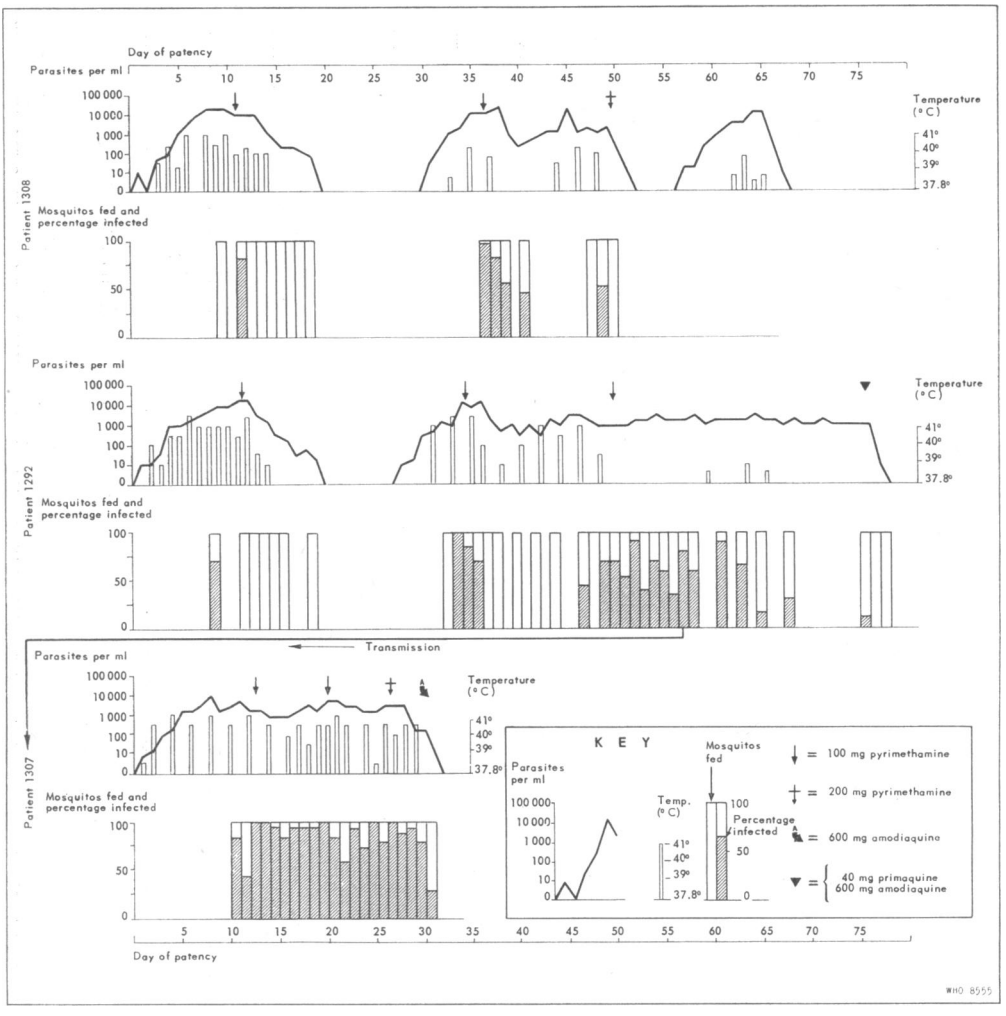
The induced infection in patient 1252 was treated with 25 mg of pyrimethamine. The response was poor; on the eighth day after treatment, 41% of the parasites remained (Table 1). On that day a 50-mg dose was given without any appreciable effect on the parasitaemia during the following week.

TABLE 2. INFLUENCE OF SINGLE DOSES OF PYRIMETHAMINE UPON PARASITAEMIA AND MOSQUITO INFECTIONS WITH *PLASMODIUM VIVAX*

Patient 1292 (Chesson)					Patient 1308 (Chesson)					Patient 1307 (Chesson)				
drug day	parasites per ml	average number of oocysts	gut infection (%)	gland infection (%)	drug day	parasites per ml	average number of oocysts	gut infection (%)	gland infection (%)	drug day	parasites per ml	average number of oocysts	gut infection (%)	gland infection (%)
—3	14 689	10	90	56						—2	7 140	15	90	75
—1	24 200	0	0		—2	22 494	0	0		—1	3 725	3	80	0
0[a]	14 744	0	0		0[a]	21 423	13	100	70	0[a]	3 060	98	100	100
1	5 712	0	0		1	16 319	0	0		1	1 787	75	100	100
2	1 873	0	0		2	1 850	0	0		2	1 586	64	100	80
3	390	0	0		3	820	0	0		3	1 785	61	90	80
4	260	0	0		4	350	0	0		4	2 230	126	100	80
5	50				5	350	0	0		5	4 800	147	100	80
6	70				6	120	0	0		6	2 677	71	100	80
7	20	0	0	0	7	80	0	0		7[a]	7 712	222	100	100
8	0				8	0				8	6 788	124	90	80
15	0				18	0				9	4 285	121	60	60
16	10				19	60				10	5 040	91	100	80
17	30				20	200				11	3 060	50	80	60
18	470				21	920				12	3 400	77	100	100
19	630				22	1 830				13	4 080	9	80	80
20	2 160				23	11 246				14[b]	5 580	69	100	100
21	12 241	0	0	0	24	—				15	6 120	47	90	80
22	18 337	455	100	100	25[a]	8 880	140	100	87	16	280	53	100	80
23[a]	16 319	27	82	100	26	2 172	52	70	100	17[c]	280	200	80	80
24	20 051	52	70	50	27	1 440	17	40	100	18	10	2	30	20
25	2 954	0	0		28	240				19	0			
26	870	0	0		29	460	4	40	50	20	0			
27	1 220				30	700				21	0			
28	450	0	0		31	1 428				22				
29	1 477				32	990				23				
30	400	0	0		33	3 150				24				
31	2 255				34	1 260								
32	1 639	0	0		35	2 760								
33	5 355				36	1 080	0	0						
34	4 285				37[b]	2 448	1	50	10					
35	3 427	3	50	36	38	220	0	0						
36	1 477				39	30	1	5						
37[a]	1 521	11	80	50	40	0	0	0						
38	1 030	15	80	86	41	0								
39	890	4	67	55	42	0								
40	2 197	18	100	67	43	0								
41	2 010	25	50	29	44	0								
42	3 990	22	100	46	45	20								
43	1 714	10	80	40	46	30								
44	2 596	6	50	21	47	230								
45[d]	1 947	9	80	83[d]	48	790								
46	3 895	10	83	36	49	—								
47	1 714				50	5 580								
48	3 120				51	6 010	29	100	100					
49	2 856	4	90	90	52	15 317	29	100	80					
50	3 520				53	8 990	2	80	80					
51	1 979	2	70	40	54	560	0	0						
52	4 080				55	10	0	0						
53	1 862	1	30	0	56	0								
54	2 380				57	0								
55	1 350				58	0								
56	2 315	1	50	15	59	0								
57	1 624													
58	1 428													
59	2 140													
60	960													
61	1 170													
62	1 560													
63	1 428													
64[e]	1 382	2	20	0										
65	10	0	0	0										
66	0	0	0	0										
67	0													
68														

[a] 100 mg pyrimethamine after mosquitos fed
[b] 200 mg pyrimethamine after mosquitos fed
[c] Amodiaquine 600 mg
[d] Sporozoite transmission to patient 1307 after preservation at —78°C
[e] Amodiaquine 600 mg + primaquine 40 mg

FIG. 2. DEVELOPMENT AND TRANSMISSION OF PYRIMETHAMINE RESISTANCE IN *PLASMODIUM VIVAX* (CHESSON STRAIN)



Patient 1256 was inoculated from patient 1252 before the latter received treatment. When patient 1256 was treated with 25 mg of pyrimethamine, the response was poor. The parasites did not disappear until chloroquine treatment was given 40 days later. The resistance to the drug which had developed in one patient (1234) had been transmitted through a second patient (1252) without further contact with the drug, and was still evident, apparently undiminished, in the third patient (1256). The length of time from the first to the second contact with the drug was 140 days.

Two infections (patients 1292 and 1308; Table 2 and Fig. 2), relapsing after an initial 100-mg dose, were not eliminated from the blood-stream

upon receiving a second 100-mg dose, thus showing a poorer response to the drug than initially. One of these (patient 1292, Tables 1 and 2) was treated a third time with 100 mg two weeks later without eliminating the erythrocytic parasites. Twenty-seven days later 600 mg of amodiaquine and 40 mg of primaquine in a single dose eliminated the parasites within 48 hours.

Mosquitos fed upon patient 1292 after the third dose of pyrimethamine became infected. Sporozoites from mosquitos infected eight days afterwards were preserved at −78° C for 161 days and injected into patient 1307. On the 13th day of patency this patient was given 100 mg of pyrimethamine, representing the fourth consecutive contact of this linear strain of parasites with the drug. The parasites, far from being eliminated, had more than doubled in number seven days later (Table 2). Another dose was given, the fifth consecutive contact of the parasites with 100 mg of pyrimethamine. The parasite density was reduced only slightly. On the 30th day of the infection, after the patient had experienced 20 paroxysms, which is about the normal course of an untreated primary infection, a dose of 200 mg of pyrimethamine was given, twice the preceding dose. The parasites were still present after 48 hours when, because of the physical condition of the patient, it was necessary to terminate the malaria. He received a single dose of 600 mg amodiaquine, which cleared the blood-stream in 48 hours (Table 2).

The sporontocidal effect of the drug paralleled the schizontocidal effect. At the first contact with the drug, the sporontocidal effect was very rapid, becoming apparent within two hours, as evidenced by a reduction in the number of oocysts. When 25 mg of pyrimethamine were given, the sporogonous cycle was completely interrupted in mosquitos feeding 8 hours after the drug administration (Young & Burgess, 1957). When 100 mg were given (patient 1308), the sporogonous cycle was interrupted within 4 hours. However, at the second contact with the drug the sporontocidal effect was less rapid; 4 days following the drugging, about half the mosquitos were still being infected (Tables 2 and 3, Fig. 2). In another case (1292), the second dose exerted virtually no effect during the first 20 hours but the sporogonous cycle was completely inhibited after 48 hours (Table 3).

A third dose of 100 mg given to patient 1292 had virtually no effect on the sporogonous cycle. Sporozoites from mosquitos infected shortly after the third dose of drug were preserved at low temperatures and later transmitted the infection to patient 1307. Two 100-mg doses of pyrimethamine, representing the fourth and fifth contact with the drug by this parasite line, were given a week apart to patient 1307 with virtually no sporontocidal effect. A 200-mg dose (the sixth drug contact) given a week after the second 100-mg dose also had little, if any, sporontocidal effect for the three days immediately after the drug. The patient then received 600 mg of amodiaquine.

TABLE 3. SPORONTOCIDAL ACTIVITY OF PYRIMETHAMINE UPON *P. VIVAX* (CHESSON)

Patient	Quantity of pyrimethamine (mg)	Percentage of mosquitos infected [a] hours before (—) and after (+) drugging											
		−24	−4 to 0	+2	+4	+8	+12	+16	+20	+24	+48	+72	+96
1308	100	—	70	—	0	0	0	0	0	0	0	0	0
	100	87	—	—	—	—	—	—	—	100	100	—	50
	200	10	—	—	—	—	—	—	—	0	—	0	—
1292	100	0	0	0	0	0	0	0	0	0	0	0	0
	100	100	100	—	100	82	70	90	70	50	0	0	—
	100 [b]	—	50	—	—	—	—	—	—	86	55	67	29
	[c]	—	0	40	30	0	—	0	—	—	0	—	—
1307	100	—	100	—	100	100	100	90	100	100	80	80	80
	100	100	—	—	—	—	—	—	—	80	60	80	60
	200	100	—	—	—	—	—	—	—	80	80	80 [d]	—
	[e]	80	80	—	100	100	—	—	—	20	—	—	—

[a] Percentage of glands with sporozoites, except when all guts had been negative in which case no glands were dissected; — = no data

[b] Transmitted to patient 1307 on eighth day after third treatment

[c] Amodiaquine 600 mg + primaquine 40 mg

[d] Received amodiaquine 600 mg on this day after mosquitos fed

[e] Amodiaquine 600 mg

Discussion

In these experiments, the first contact of the parasites with a single, normal dose of pyrimethamine resulted in their slow elimination from the blood-stream. All observed cases relapsed. In every case the response of the parasite to the second dose of drug was less marked than the first; in one case, the drug had no appreciable effect even though the dosage was twice as high as the first. Subsequent doses of pyrimethamine had virtually no effect on the parasitaemias or clinical courses of the disease.

When this resistant malaria was induced in a recipient by either blood transfusion or mosquito bite, the resistance was still evident. When challenged by the drug again, the parasites responded as if they were being challenged another time in the donor host. In some, but not all, cases it appeared that resistance increased with each contact with the drug.

Asexual and sexual parasites manifest resistance simultaneously. The resistant parasites can be transmitted by mosquitos or by blood transfusion. The resulting infections in the recipients show the same degree of drug resistance as those of the donor.

Hernandez et al. (1953) developed pyrimethamine resistance in the Chesson strain of *P. vivax* by exposing the parasite to subcurative daily doses over prolonged periods of time. They concluded that resistance would come slowly in the field if adequate doses were employed and indicated that it was doubtful whether resistance would become a serious problem under field conditions.

The evidence presented in the current report indicates that resistance to pyrimethamine can develop in *P. vivax* parasites after treatment with large single doses of the drug and that it appears quickly, being manifested in varying degrees by the re-appearing parasites to the second challenge by the drug. In some of the relapsing cases which exhibited resistance, the second dose was given less than a month after the initial treatment. In field work, where the drug is given to large populations at monthly or less frequent intervals, it seems logical to assume from the above experience that resistance by *vivax* malaria may appear just as has been reported in several places for *P. falciparum*. It may be possible for resistance by *vivax* to occur even if the drug is given oftener than every month. Clyde & Shute (1957) reported that *P. falciparum* developed resistance when weekly doses were given.

The resistant character appears to be durable. It was transmitted by erythrocytic parasites and by sporozoites, both preserved at −78° C, without apparent change. The resistance was still evident 140 days after the initial contact with the drug, during which time the infection had passed through a second patient without contact with the drug, and then into a third where the challenge was made. This suggests persistence of the characteristic without frequent exposure to the drug.

RÉSUMÉ

Dès les premiers essais de traitement de l'infection à *Plasmodium* par la pyriméthamine, il est apparu que le parasite développait une certaine résistance au médicament. La réponse au second traitement, lors d'une rechute, était moins nette que lors du premier traitement. Des essais contrôlés ont été effectués pour étudier cette résistance.

L'effet de la pyriméthamine a été évalué sur des malades atteints de neurosyphilis, infectés par *P. vivax*, et traités par la pyriméthamine. Dix-sept malades ont reçu des doses uniques de 25, 50, 100 ou 200 mg respectivement. Le premier traitement de 25 ou de 100 mg a été administré entre le 8e et le 61e jour de la période de patence. Il a exercé une action schizontocide modérée et une action sporontocide rapide. Tous les cas traités ont présenté des rechutes.

Le second traitement, administré trois semaines ou plus après le premier, avec les mêmes doses ou des doses supérieures, a eu soit un effet atténué soit aucun effet sur les cycles schizogonique ou sporogonique. Des traitements ultérieurs, à intervalles hebdomadaires, n'ont eu aucun effet. La résistance du parasite s'est maintenue, que le parasite ait été transmis à un nouvel hôte par piqûre de moustique, injection de sporozoïtes conservés ou par transfusion de sang infecté. La conservation des formes érythrocytaires à −78° C n'a pas affecté sensiblement leur résistance.

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