THE USE OF LONG ACTING SULFONAMIDES, ALONE OR WITH PYRIMETHAMINE, IN MALARIA (WITH SPECIAL REFERENCE TO SULFORMETOXINE) *

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The antimalarial activity of the sulfonamides was described very soon after the discovery of these drugs. As early as 1940, a number of papers, such as those by Diaz de León (31-33), Hill and Goodwin (56), Van der Wielen (129), Coggeshall (18-20, 23), Niven (83), Chopra et al. (10-13), Menk and Mohr (81), Farinaud et al. (37-38), Sorley and Currie (118), Sinton et al. (117), etc., had reported on a somewhat variable success obtained with sulfonamides in experimental and human malaria. For a further 10 years, i.e. until 1950, there still existed some interest in this chemical group, as reflected in some important clinical trials, for instance those by Fairley et al. (36) and by Coatney et al. (16, 17). However, with the advent of more reliable synthetic antimalarials, chemotherapeutic work on sulfonamides with practical aims was almost dropped and rather remained limited to basic questions of more theoretical interest like mechanism of action, potentiation of the effect of other antimalarials of the folic-acid-antagonist group on experimental malaria, cross resistance, etc. This work is linked to renowned names such as Greenberg, Rollo, Goodwin, Bishop, Eyles and Coleman, Hitchings, etc.

Excellent cumulative reviews on sulfonamides and malaria have been made by Curd (27) in 1943, Findlay (42) in 1951, and Hill (58) in 1963.

It is somewhat astonishing that no major work was carried out during the years from 1955 to 1963, i.e. during the period in which, thanks to the discovery of the so-called long-acting sulfonamides , a better knowledge of the pharmacokinetics of these drugs was obtained. It is now evident that many of the early contradictory reports regarding the antimalarial effect of sulfonamides, made during the initial years of the sulfonamide era, were due to the scanty information on the pharmacokinetics of these substances in man and in laboratory animals, a gap which led or misled, among other things, towards empirical and therefore not quite reliable dosage.

At present, the reported resistance of certain *Plasmodia* strains to some major antimalarials such as pyrimethamine, chloroquine and perhaps even quinine, and, on the other hand, the availability of some sulfones and sulfonamides with a very sustained action, have made it advisable to reexamine the possible value of these substances as an auxiliary tool for the management of malaria. It is most unfortunate that this logical interest is hampered by the reports on cases of Stevens-Johnson and Lyell syndrome, observed during the use of some of these substances.

The purpose of this short review is to summarize: a) the available infor-

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mation on the effect of the sulfonamides on the different species and cycle stages of *Plasmodia*, b) their mechanism of action and its implications, i.e.: potentiation and resistance, and c) the trials so far carried out with the longest acting sulfonamide sulformetoxine, Ro 4-4393 *.

As it can be seen from Tables I to IV, the effect of the sulfonamides has been tested on the blood schizonts in most of the laboratory animal *Plasmodia*. They are considerably active against the blood schizonts of *P. berghei*, *P. gallinaceum*, *P. knowlesi* and *P. falciparum* and less or not active in the rest. The results obtained in cases of spontaneous or induced *falciparum* malaria have been almost unanimously positive although the onset of action of the sulfonamides is slower than that of the major antimalarials. This has precluded their use in the treatment of acute malaria. The results reported on malaria due to *P. vivax* are less uniform. Some authors find them to be effective here (although always less so than in *P. falciparum*), whereas others (35, 36, 51, 81, 93, 106) find practically no therapeutic activity. This may have been due to differences in the dosage or to the fact that the effects of different sulfonamides vary considerably (42). Very few investigations have been carried out with sulfonamides in cases of *malariae* and *ovale* malaria.

Sulfonamides have a definite causal prophylactic effect against certain *Plasmodia* such as *P. knowlesi* and *P. gallinaceum* (Table II). Regarding human malaria, the reports are rather scanty, but it seems that sulfonamides, at the doses tested, do not act as causal prophylactics (16, 17).

The sulfonamides do not exert any effect on the gametocytes of the human types of malaria (Table III). In fact, they can even increase their number in the blood (41).

Fairley et al. in 1945 and Findlay et al. in 1946 reported that sulfamezathine rendered the gametocytes of *P. falciparum* incapable of developing in the mosquito (Table IV). However, Laing in his recent studies did not find such an effect with Fanasil (70). Further research on this subject is therefore needed. There is no information which would allow the assumption that sulfonamides possess any activity as secondary tissue schizonticides in human malaria. According to Bishop, exo-erythrocytic parasites are "relatively insensitive" to proguanil and sulfadiazine (7).

Mode of action of sulfonamides in malaria: It is paradoxical that the mechanism of action of sulfonamides, although not in all its details, is better known than that of most major antimalarials. Since the initial work by Maier and Riley (76), who proved in 1942 that the antimalarial effect of the sulfonamides is antagonized by p-aminobenzoic acid, a number of studies have been carried out, mainly by Greenberg, Goodwin, Rollo, Bishop, Hitchings, Thurston, etc. (6, 47, 49, 50, 59, 60, 101, 102, 127). Sulfonamides, biguanides and pyrimethamine constitute the group of the so-called folic-acid-antagonist antimalarials, i.e. "those antimalarials whose action has been considered to be concerned with interference with the synthesis of purines and pyrimidines via the PAB → folic acid → folinic acid system" (102). According to Rollo (Fig. 1), sulfonamides probably act on reaction A by simple metabolite competition, whereas biguanides and pyrimethamine act in a more complicated manner on reaction B. These analogies and differences between the mechanism of action of sulfonamides on the one hand and of the biguanides and pyrimethamine on the other entail a complex framework of possible reciprocal effects when two of these drugs are given together (potentiation) or in succession (cross resistance).

The possibility of cross resistance has been dealt with in several recent

(*) Trade name: Fanasil Roche (also mentioned in the literature as sulforthomidine and sulforthodimethoxine).

Table I

Blood schizonticidal activity

Sulfonamides have been found active as blood schizonticides in:

Parasite	References
P. berghei	29,57-58,82,94,98-100,124-125
P. gallinaceum	28,58,78,94,119,132
P. lophurae (less sensitive than P. gallinaceum)	21,28,78,79
P. fallax (less sensitive than P. gallinaceum)	103
P. circumflexum (less sensitive than P. gallinaceum)	77
P. knowlesi	10-11,18-23,92,95
P. cynomolgi (less sensitive than P. knowlesi	21-23,53,116
P. inui (less sensitive than P. knowlesi)	20,21,23
P. falciparum	2,12-13,16,23,33,36-38,41,56, 66-69,71,73,75,81,83,89,106, 110,117,132
P. vivax (less sensitive than P. falciparum)	12-13,17,23,37-38,56,61,73, 118,132
P. malariae (less sensitive than P. falciparum)	12-13,23,26,37,45,110,129,132

Sulfonamides have been found inactive as blood schizonticides in:

Parasite	References
P. lophurae	18
P. relictum	27,29,57,77
P. cathemerium	18,28,77,132
P. nucleophilum	77
P. vivax	35-36,51,81,93,106

BEST CLASSICAL BLOOD SCHIZONTICIDAL DRUGS: 4-AMINOQUINOLINES
MEPACRINE, QUININE, PYRIMETHAMINE, PROGUANIL

OF THE ABOVE UNPRACTICAL FOR SUPPRESSION: MEPACRINE, QUININE
UNPRACTICAL FOR TREATMENT: PYRIMETHAMINE, PROGUANIL

Table II

Causal prophylaxis

Sulfonamides have been found active as causal prophylactics in:

Parasite	References
P. knowlesi	42
P. cynomolgi (less sensitive than P. knowlesi)	53
P. gallinaceum	15,24,28-29,41-43,112,130
P. cathemerium* (canary) (less sensitive than P. gallinaceum)	132
P. lophurae (turkey) (less sensitive than P. gallinaceum)	132
P. falciparum	117

Sulfonamides have been found inactive as causal prophylactics in:

Parasite	References
P. falciparum	16,36,132
P. vivax	16,36
P. relictum	77
P. nucleophilum	77
P. cathemerium* (canary)	18,28,132
P. lophurae (chicks and ducklings)	18,28

* Some sulfonamides active; some others inactive

BEST CLASSICAL CAUSAL PROPHYLACTIC DRUGS: PROGUANIL AND PYRIMETHAMINE (P. FALCIPARUM > P. VIVAX)

ACTIVE BUT NOT PRACTICAL: 8-AMINOQUINOLINES

Table III

Gametocytocidal activity

Sulfonamides have been found inactive as gametocytocides in:

Parasite	References
P. falciparum	14,33,36,42,70,111
P. vivax	42
P. malariae	42

BEST CLASSICAL GAMETOCYTOCIDAL DRUGS: 8-AMINOQUINOLINES

ACTIVE ONLY IN P. VIVAX AND P. MALARIAE: 4-AMINOQUINOLINES, MEPACRINE, QUININE

reviews (7, 58, 107-109). Fig. 2 to 9 are a poor attempt to represent graphically the main observation of cross or coincidental resistance in experimental malaria due to *Plasmodia berghei, gallinaceum, cynomolgi* and *knowlesi* that have been reported. These figures make no claim to being complete or perfect since I may have missed some valuable reports. On the other hand, there are some minor discrepancies in the literature, undoubtedly due to differences in the techniques used for creating resistance and to the inevitable differences among the strains.

Nevertheless, with regard to any possible clinical application of sulfonamides (alone or combined with pyrimethamine) the most important facts about experimental cross resistance are:

1) Sulfonamide-resistant strains of some species of *Plasmodia* (e.g. *P. gallinaceum*) are usually also resistant to proguanil and pyrimethamine (7, 101).
2) Strains resistant to pyrimethamine and proguanil or its triazine derivative are generally not or only partially resistant to sulfonamides (85, 98, 101, 113, 115, 122, 126).
3) DDS-resistant strains are usually resistant to sulfonamides (8, 87-88, 90, 122).
4) Usually there is no cross resistance between sulfonamides or sulfones and antimalarials not belonging to the group of the folic-acid-antagonists. Frequently there is even some hypersensitivity (55, 63, 84-88, 91, 120, 122).

Table IV

Sporontocidal activity

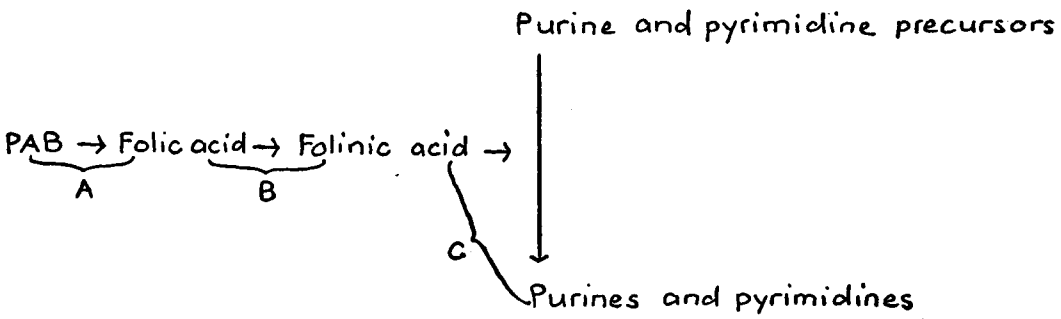
Sulfonamides have been found active as sporontocidal drugs in:

Parasite	References
P. falciparum	36,42

Sulfonamides have been found inactive as sporontocidal drugs in:

Parasite	References
P. falciparum	70

BEST CLASSICAL SPORONTOCIDAL DRUGS: PYRIMETHAMINE, PROGUANIL
ACTIVE BUT LESS PRACTICAL: 8-AMINOQUINOLINES



after Rollo (102)

Fig. 1

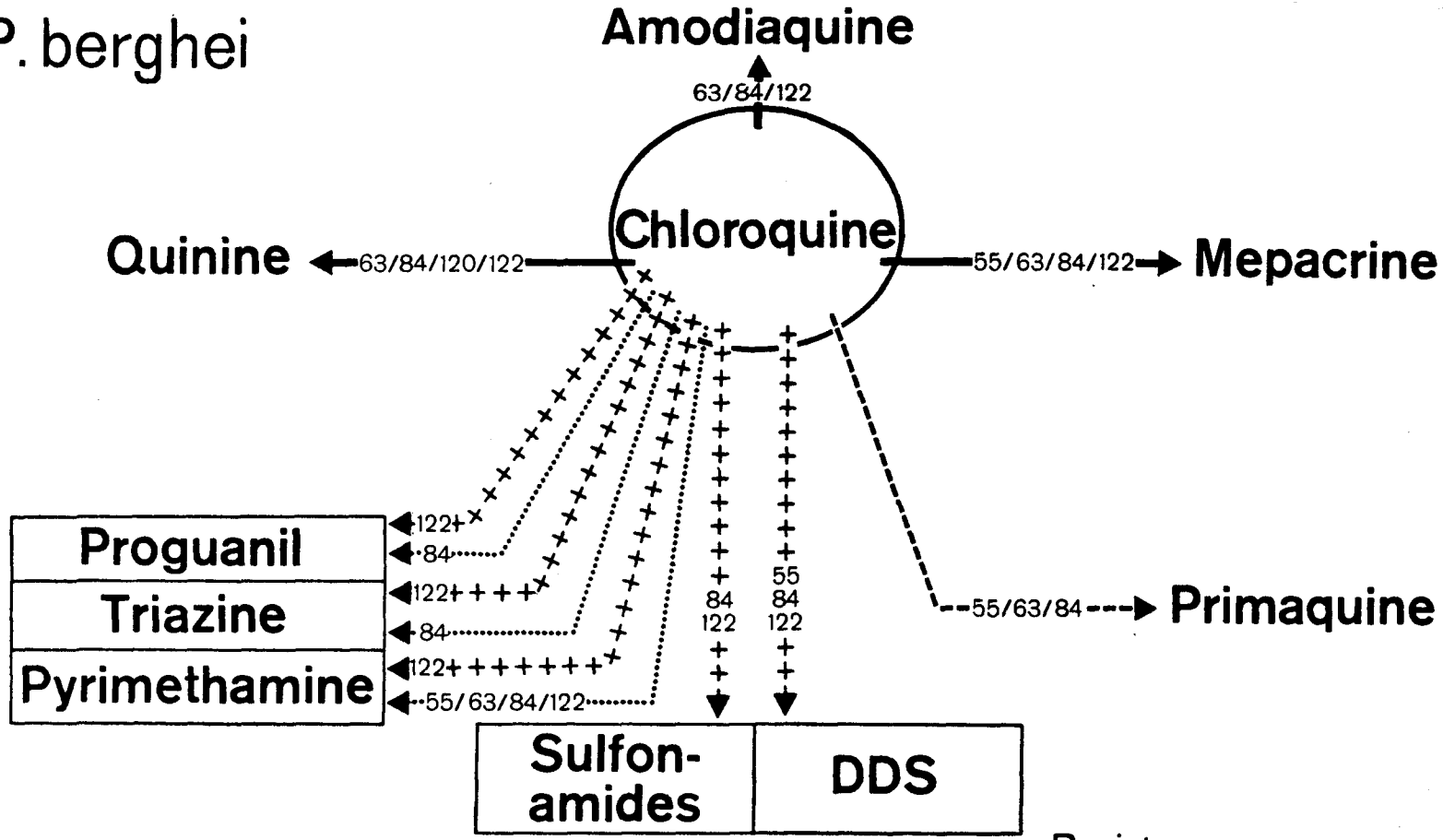


Fig. 3: P.berghei resistant to **4-aminoquinolines.** Cross resistance to other antimalarials. (The arrows go from the substance to which the strain has been made resistant to the substance tested.)

P. berghei

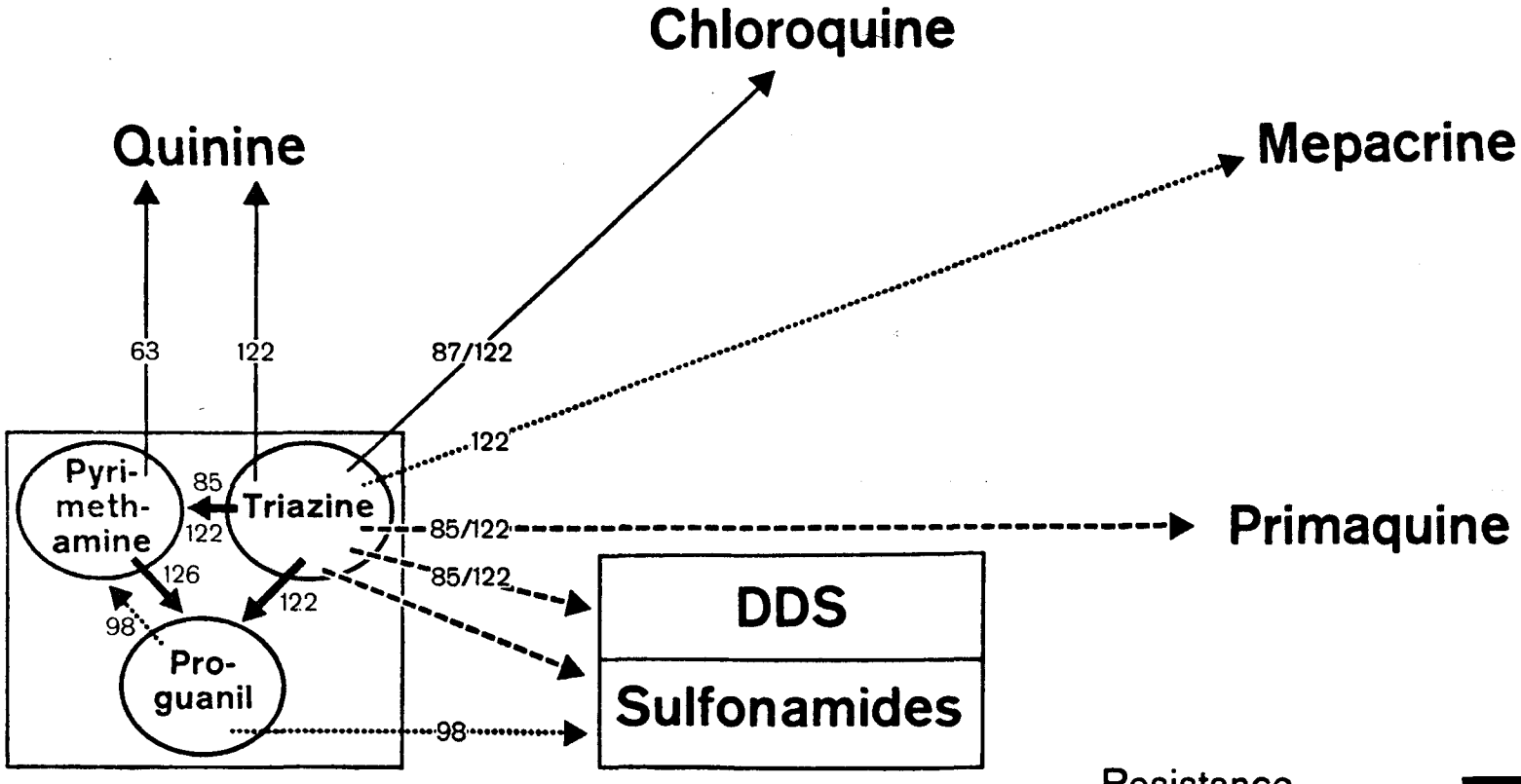


Fig. 4: P. berghei resistant to the group **Proguanil-Triazine-Pyrimethamine.** Cross resistance to other antimalarials. (The arrows go from the substance to which the strain has been made resistant to the substance tested.)

P. berghei

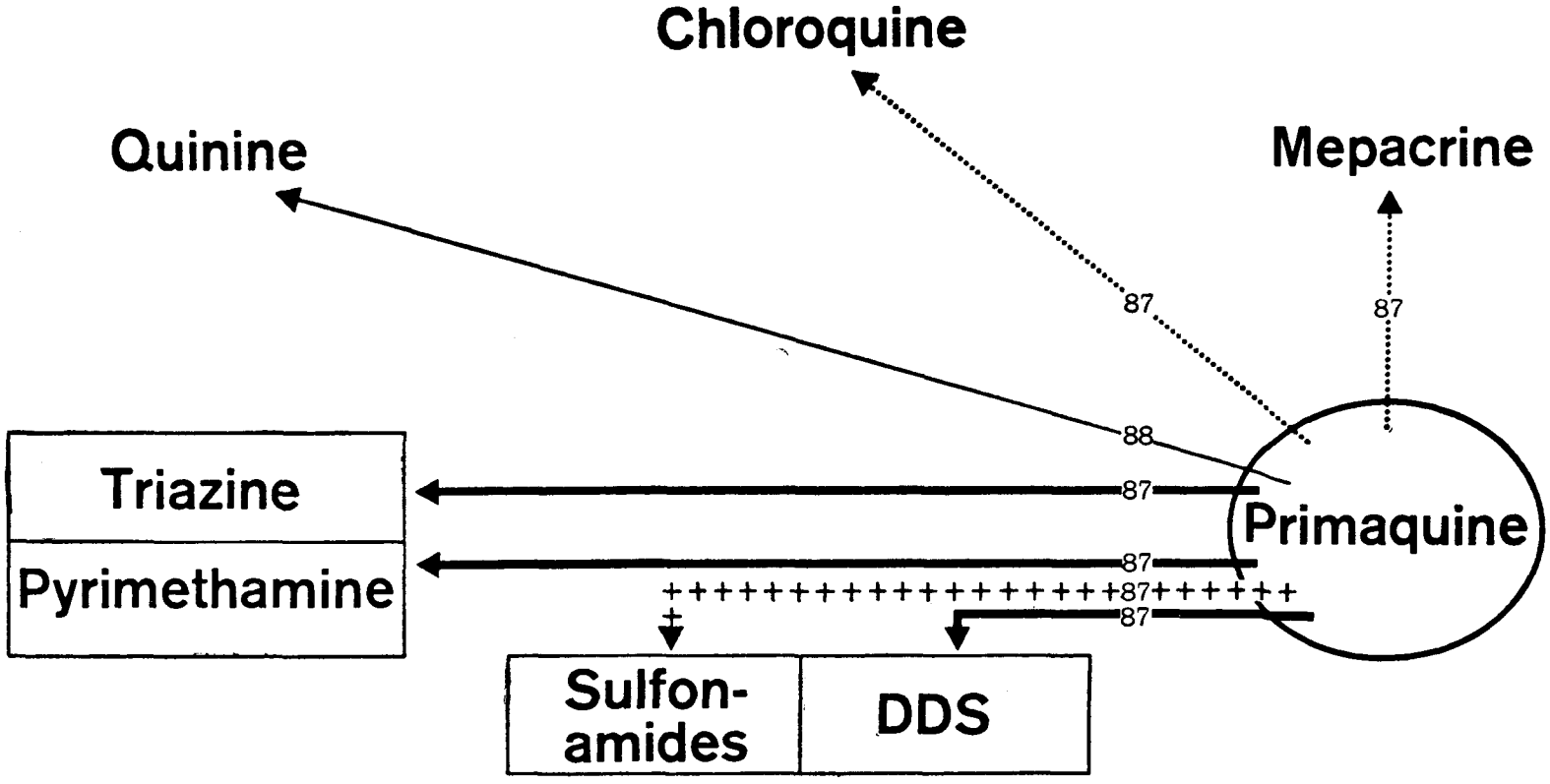


Fig. 5: P. berghei resistant to the group **Primaquine.** Cross resistance to other antimalarials. (The arrows go from the substance to which the strain has been made resistant to the substance tested.)

Resistance
Slight resistance
No resistance
Hypersensitivity ++++++

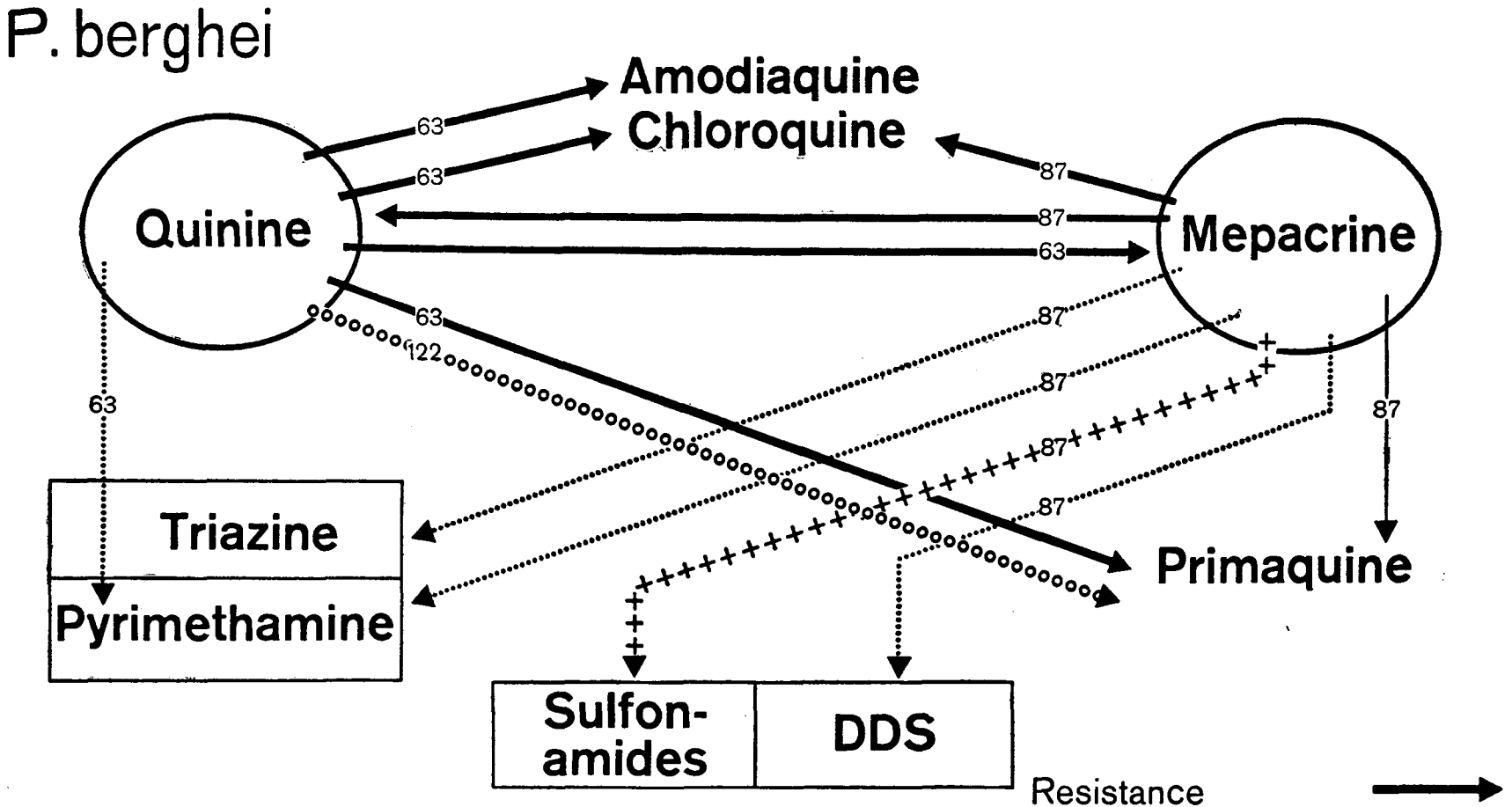


Fig. 6: P. berghei resistant to the group **Quinine or Mepacrine.** Cross resistance to other antimalarials. (The arrows go from the substance to which the strain has been made resistant to the substance tested.)

P. gallinaceum

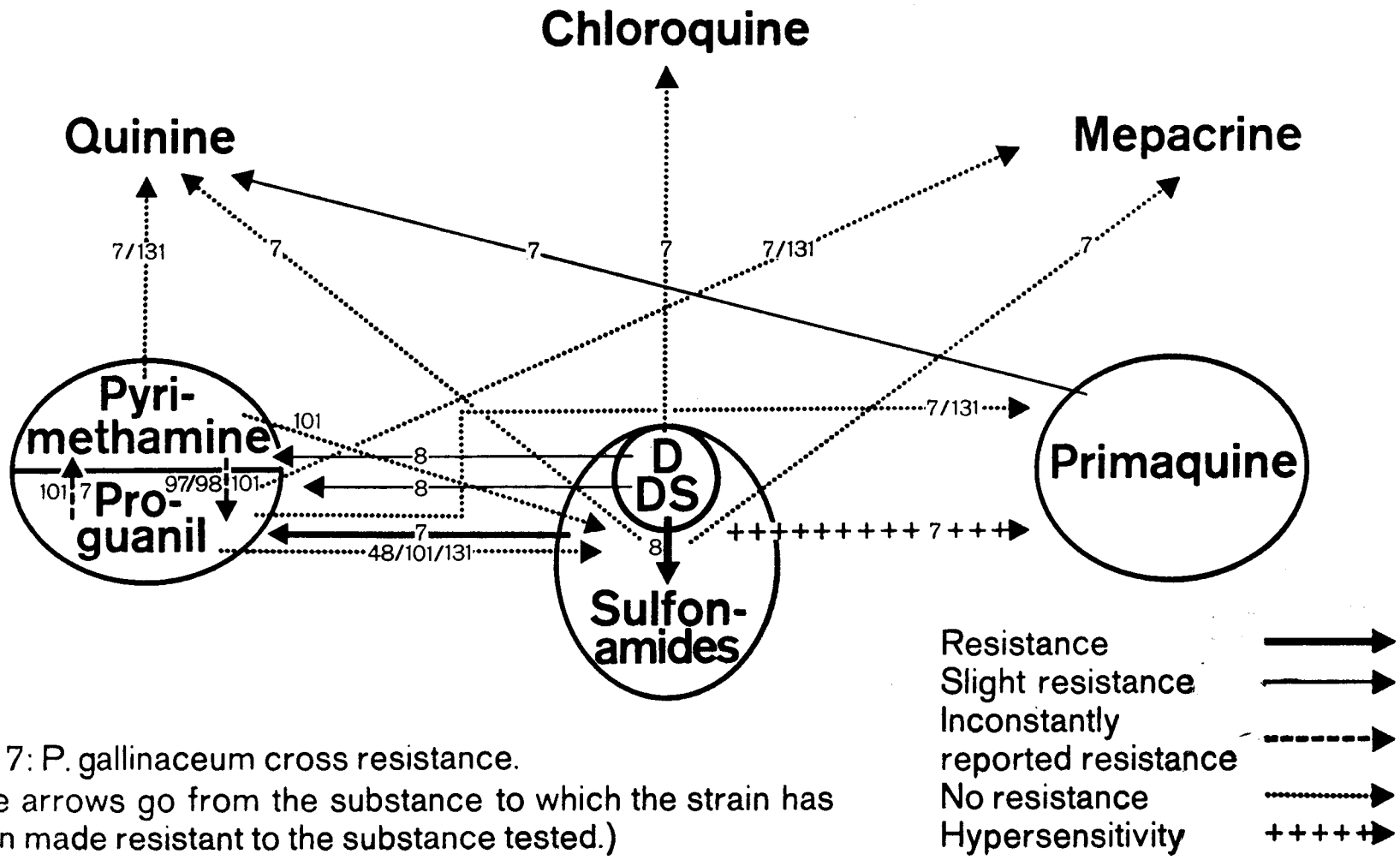


Fig. 7: P. gallinaceum cross resistance.
(The arrows go from the substance to which the strain has been made resistant to the substance tested.)

P. knowlesi

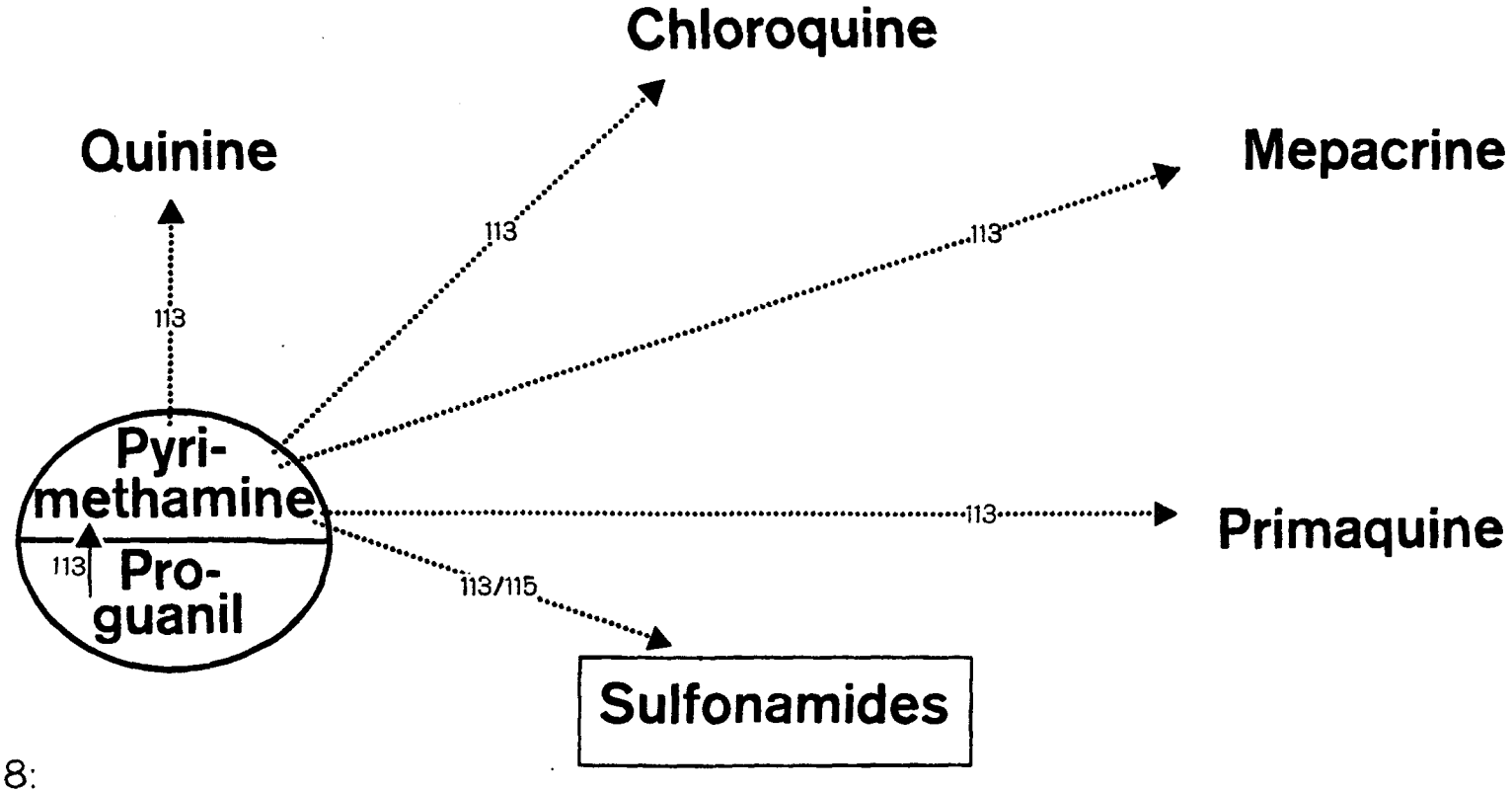


Fig. 8:
P. knowlesi cross resistant to the group **Pyrimethamine-Proguanil.**
(The arrows go from the substance to which the strain has been made resistant to the substance tested.)

Slight resistance ——→
No resistance ·····→

P. cynomolgi

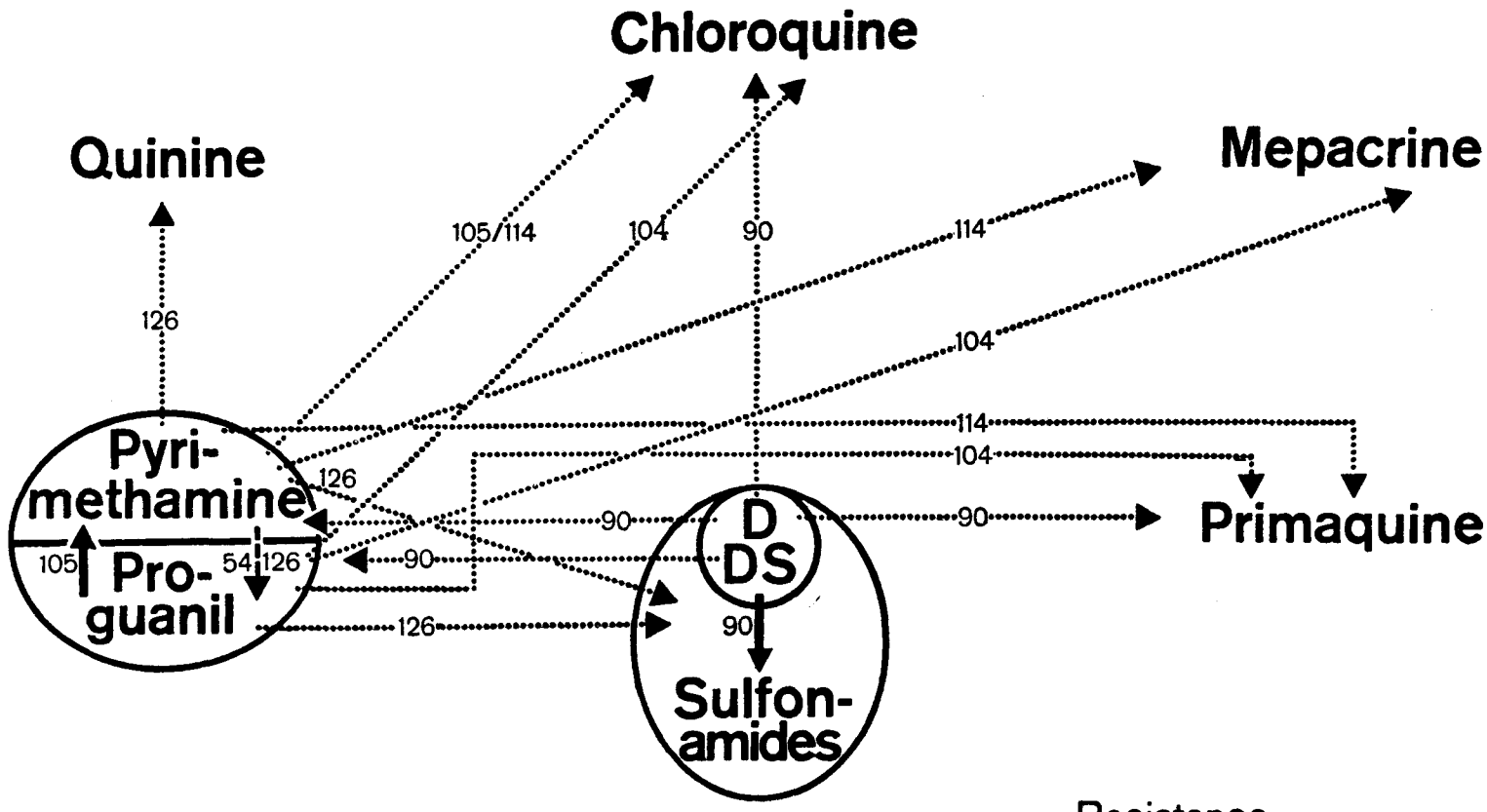


Fig. 9: P. cynomolgi cross resistance.
(The arrows go from the substance to which the strain has been made resistant to the substance tested.)

Now to potentiation. The fact that sulfadiazine and other p-aminobenzoic competitors with antimalarial activity are able to potentiate the action of proguanil against blood schizonts of *P. gallinaceum* has been known since the work by Greenberg in 1949 (47, 49-50). Proguanil and pyrimethamine do not potentiate each other (101). Later on, it was proved that the same applies if the sulfonamide is replaced by a sulfone and/or if proguanil is replaced by pyrimethamine (4-5, 101). A similar potentiation has in the meantime also been confirmed in experimental and human toxoplasmosis (34, 44, 59). In malaria, however, there had until 1963 been only a few tentative attempts to test the potentiating effect between sulfonamides and pyrimethamine in man (Hurly in 1959 (62); McGregor, Williams and Goodwin in 1963, (80).

In 1964, DeGowin and Powell (30) showed that 2,0 g sulfadiazine daily for 5 days given concurrently with 50 mg pyrimethamine daily for 3 days cured 5 out of 6 volunteers infected with the Malaya (Camp) strain of *P. falciparum* resistant to chloroquine, hydroxy-chloroquine, quinacrine, chlorguanide, and pyrimethamine. Previous trials with the same doses of sulfadiazine and pyrimethamine given separately had not been successful.

One month later, in December 1964, Laing (67) reported on the first results of his study of the antimalarial effect of Fanasil (Ro 4-4393). This sulfonamide shows the longest period of elimination ever described in man. It has a half-life of 100 to 200 hours. (Sulfadiazine: 17 hours). This permits therapeutically sufficient blood levels to be maintained by weekly administration of low doses, either orally or parenterally. The slow elimination is not due to any intentional conventional modification of the basic molecule (e. g. N_1 — acetylation) or to the pharmaceutical presentation (e. g. repository injection or late release tablet), but to the intrinsic structure of the basic molecule itself. Oral absorption of Fanasil is as rapid as that of the usual sulfonamides. These two properties, rapid absorption and slow elimination, together with the good activity it has shown in the usual chemotherapeutic experimental tests (96, 128), have justified extensive clinical trials with the substance in the common indications of the sulfonamides (1, 25, 64), with especial attention to those in which single-dose treatment (e.g. meningococcal meningitis in epidemic developing countries (39) and long-term treatments e.g. leprosy (3), trachoma (40), systemic mycoses (74) are most indicated.

On the basis of the experience gained during clinical trials in the treatment of about 15,000 cases (some 100 of them treated continuously for several years) it can be stated that the tolerance of Fanasil is at least as good as that of the most highly reputed sulfonamides. The possibility of oral weekly administration makes Fanasil quite suitable for combined treatment with pyrimethamine.

The activity of Fanasil in experimental malaria has been studied by Richards (94) (Table V). Fanasil showed good antimalarial activity and a marked potentiation with pyrimethamine against sensitive strains as well as against strains of *P. gallinaceum* and *P. berghei* resistant to pyrimethamine, triazine or chloroquine. The pyrimethamine-resistant strain of *P. gallinaceum* was somewhat less sensitive to the potentiating mixture (pyrimethamine + Fanasil) than the normal strain. However, the resistance factor was only 2 to 5, as compared with > 300 for pyrimethamine alone.

These results have been confirmed in their main lines by Brener (9). The optimum potentiating ratio in these chemotherapeutic studies seems to be in the region of 10: 1 (Fanasil: pyrimethamine) but the range appears to be very broad. Prophylactic single-dose treatment showed that Fanasil has the most prolonged activity among other long-acting sulfonamides and DDS.

Fanasil (20 mg/kg x 7) and Fanasil + pyrimethamine (2.6 mg/kg + 0.004 mg/kg x 7) were also effective against infection produced by *P. gallinaceum sporozoites*. Treated chicks remained free from parasites for the duration of the experiment (35 days of observation), whereas the controls died within 17 days (94). It seems therefore that there may also be a potentiation of the causal prophylactic effect.

Treatment of *acute malaria* with Fanasil alone or combined with pyrimethamine:

a) *P. falciparum*: Laing (68, 71, 72), working in Amani, Tanzania, i.e. in an area where pyrimethamine resistance is known to occur, has reported the following results:

A total of 105 "semi-immune" Bantu Africans seeking treatment for fever were treated with one dose of Fanasil (1 g) alone (45 cases) or with one dose of 500 mg Fanasil in combination with 12.5 mg pyrimethamine (45 cases) or with one dose of chloroquine (200 mg i.m. or 600 mg orally) (15 cases) and thereafter a diagnosis of acute *falciparum* malaria was made by means of parasite counts from thick blood films. The results are shown in Table VI. In the group receiving Fanasil + pyrimethamine, asexual parasitemia was cleared in 88% of the patients within 48 hours compared to only 30% in the group receiving Fanasil alone. In another experience, two small children who had not responded to 50 mg DDS did respond to 250 mg Fanasil alone (69).

In an unpublished study carried out recently in Malaya. Laing (72) tried Fanasil alone or with pyrimethamine in 62 "semi-immune" patients suffering from acute malaria due to *P. falciparum*. The results are shown in Table VII. The treatment was considered to be a failure in those cases where asexual parasitemia was present on the seventh day of observation or where therapeutic intervention with another antimalarial drug was necessary in the interest of the patient. After a single dose of 1 g Fanasil given to 9 individuals there was one failure. Clearance of asexual parasitemia was somewhat slower than in African patients (average 3.1 days compared with 2.3 days). Various combinations of Fanasil (200 mg to 1 g) with pyrimethamine (25 to 50 mg) gave similar results in 53 patients (6 failures) with an average duration of parasitemia of 2.2 days. Among the few patients receiving the highest dose of 1 g Fanasil + 50 mg pyrimethamine there were no failures. 4 patients who had early recrudescences of parasitemia and febrile symptoms after treatment with chloroquine (1.5 — 2.0 g orally over 3 to 4 days in 3 adults and 0.280 g parenterally in a two year old girl) were also treated with a single dose of Fanasil + pyrimethamine (1 g Fanasil + 25 mg pyrimethamine for the adults and 250 mg Fanasil + 6,25 mg pyrimethamine for the child); three of the patients were "apparently cured" and one (an adult) to whom pyrimethamine was given 3 days prior to Fanasil showed no response to the dose of pyrimethamine, whereas after Fanasil scanty asexual parasitemia persisted for 7 days. Among 36 patients treated with 600 to 2,500 mg chloroquine there were 11 failures .

In Bangkok, Harinasuta (52) gave Fanasil alone or in combination with pyrimethamine or chloroquine to 66 patients (adults) with recrudescences after chloroquine-resistant acute *P. falciparum* malaria. The preli-

The antimalarial activity against drug sensitive and drug resistant strains of P. gallinaceum and P. berghei. Results expressed as the level of drug which reduces the parasitemia to 50% of the untreated controls (ED_{50}) (Richards[94])

Drug	ED_{50} (mg/kg x 7 orally)				
	P. gallinaceum			P. berghei	
	Pyrimethamine sensitive strain	Pyrimethamine resistant > 300 x	Triazine resistant > 25 x	Chloroquine sensitive strain	Chloroquine resistant > 12 x
Pyrimethamine	0.03	> 10	-	0.15	0.15
Sulphorthomidine	20.0	25	25	1.0	1.0
Sulphorthomidine + pyrimethamine	2.6 + 0.004	5 + 0.02	-	0.1 + 0.0125	0.1 + 0.0125
Chloroquine	-	-	-	8.0	> 100.0, toxic

Table VI

Average duration of asexual parasitaemia of P. falciparum and fever after treatment with Fanasil alone and in combination with pyrimethamine (Laing, Tanzania[71,72])

Drug	No. of Pats.	Failures*	Mean duration in days	
			Parasitaemia	Fever
Fanasil ** 1 g	45	7	2.3	2 (19 cases)
Fanasil 500 mg *** + Pyrimethamine 12.5 mg	45	0	1.8	1.5 (9 cases)
Chloroquine 200 mg i.m. or 600 mg orally	15	0	1.7	-

* Parasites still present in blood films on the 7th day or other drug required before the 7th day.

** Children up to 11 years received 250 to 750 mg

*** Children up to 11 years received 125 to 375 mg

Table VIII

Effect of Fanasil given alone or in combination with Daraprim or chloroquine to adult patients with chloroquine-resistant falciparum malaria (Harinasuta, Thailand[52])

Drug regimen (single dose)*	No. of patients treated	Successes		Failures		Average duration of parasitemia + fever in days (excl. failures)	
		cured	cleared**	C.T.E.	P.T.E.	Parasitemia	Fever
Fanasil 1 g	16	9	2	-	5	5	2.1
Fanasil 1.5 g	7	2	2	2	1	4.6	2.3
Fanasil 250 mg + Daraprim 12.5 mg	6	4	1	1	-	4	2
Fanasil 250 mg + Daraprim 25 mg	15	10	-	2	3	3.6	1.7
Fanasil 0.5 g + Daraprim 25 mg	5	1	2	2	-	4.1	0.6
Fanasil 1 g + Chloroquine 1.5 g (in 3 days)	17	13	-	2	2	4	1.5

* except for chloroquine
** with a follow-up of less than 28 days

C.T.E. = complete temporary effect (clearance of asexual parasitemia with subsequent relapse)
P.T.E. = partial temporary effect (reduction not abolition of asexual parasitemia)

minary results are shown in Table VIII. On the basis of clinical response and duration of parasitemia, the best results were obtained with the following combinations: A) 1 g Fanasil (single dose) + 1.5 g chloroquine in three days); B) 0.5 g Fanasil + 25 mg pyrimethamine in a single dose. The latter has the practical advantage of being a one-dose therapy. Since duration of parasitemia is longer than that seen after chloroquine in chloroquine-sensitive cases, further trials are being carried out with higher doses of the combination Fanasil + pyrimethamine.

Sensitivity to chemotherapy of seven strains of *P. falciparum* found in four Brazilian regions, in patients who did not respond satisfactorily to chloroquine, was studied by Lopes and Rodrigues da Silva (75) in 25 neurosyphilitic patient with blood-induced malaria. All seven strains showed resistance to the standard "field--test" dose (10 mg/kg of chloroquine recomended by WHO for preliminary selection of suspected chloroquine-resistant strains). Three of the seven strains turned out to be only partially chloroquine - resistant, since parasitemia disappeared after a higher dose of chloroquine (3 g in 3 days). The other four strains were considered as fully resistant to chloroquine; two of them were also resistant to pyrimethamine, and at least partially, to quinine. These two last strains (Po_2 and Po_3), i. e., those fully or partially resistant to the three above mentioned major antimalarials, were sensitive to Fanasil combined with pyrimethamine. As shown in Table IX, the therapeutic response (8 cases), however, took place rather slowly. Clinical response was achieved in 4 to 8 days, the trophozoites disappeared from the blood in 4 to 6 days and the gametocytes in about a month.

Almeida, Brazil, (2) has reported on the preliminary results of an extensive study being carried out in cases of spontaneous malaria. The patients are followed up for 6 days after receiving one of the three following treatments: Group A) 10 mg/kg chloroquine, 5 days later 10 mg/kg chloroquine and 5 mg/kg chloroquine on each of the 2 following days (total dose 30 mg/kg in 8 days). Group B) 40 mg/kg chloroquine in 4 days. Group C) 1 g Fanasil and 50 mg pyrimethamine on the first day and 0,5 g Fanasil on the following day. According to the preliminary results (197 courses of treatment in 178 patients followed), some cases of the groups A and B have shown recrudescences during the follow-up period. In the group C (14 cases already observed over 60 days) 4 cases became negative during the first day of treatment and the other 10 cases during the second one. All of them remained negative over the follow-up period.

Peringle and Lane (89), Tanzania, have reported on the results obtained with 250 mg Fanasil in 9 schoolchildren who developed one or two clinical fits of *falciparum* malaria three to six months after the injection of the repository antimalarials cycloguanil pamoate, or DADDS, or both (Table X). By the fourth day after the dose of Fanasil trophozoites had disappeared from the blood in all cases.

b) *P. vivax*. Confirming the results obtained by previous workers with older sulfonamides, Laing in Malaya also found *P. vivax* to be less sensitive to Fanasil than *P. falciparum* (Table VII) (72). Out of 13 patients with acute *vivax* malaria treated with 1 g.

Table IX

Therapeutic response to the association

Ro 4-4393 + Pyrimethamine

(Lopes and da Silva, Brazil[75])

No. of patient	Ro 4-4393/2 + pyrimethamine	Clinical response	Parasitemia	
			Pft	Pfg
18	500 mg + 50 mg 5 d	5 d	5 d	30 d
13	Idem	5 d	4 d	30 d
21	Idem	6 d	5 d	28 d
8	1 g + 50 mg 3 d	5 d	4 d	11 d*
25	Idem	8 d	5 d	24 d*
26	Idem	4 d	4 d	8 d*
27	Idem	5 d	5 d	7 d*
19	Idem	5 d	6 d	17 d*

* under observation

Pft = trophozoites (P. falciparum)
Pfg = gametocytes (P. falciparum)
d = days

Table X

Results obtained with a single dose of 250 mg Fanasil in 7 patients having developed clinical falciparum malaria 3 to 6 months after the injection of a repository antimalarial (cycloguanyl pamoate, diacetyldiaminodiphenylsulfone or both)

(Pringle and Lane, Tanzania[89])

Subject code number:	Age in years	Repository drug	Dose of rep.drug in mg/kg.	Interval after injection of rep. drug:	Trophozoite counts per cu. mm. on post-treatment days				
					Pre-treatment:	Day 1	Day 2	Day 3	Day 4
BB/10	12	Cl-501	10.8	D + 85	7.440	Neg.	Neg.	Neg.	Neg.
AB/ 4	9	"	12.4	D + 137	17.200	65	Neg.	Neg.	Neg.
AB/21	10	"	11.8	D + 179	80.360	32	112	48	Neg.
AY/27	8	Cl-556	8.4	D + 148	8.400	14.520	Neg.	Neg.	Neg.
"	"	"	"	D + 199	2.880	3.360	Neg.	Neg.	Neg.
AY/17	8	"	7.0	D + 200	44.560	4.800	2.500	3.240	Neg.
AG/ 5	9	Cl-564	14.3	D + 140	8.800	820	Neg.	Neg.	Neg.
"	"	"	"	D + 196	21.600	1.640	Neg.	Neg.	Neg.
AG/26	8	"	13.2	D + 172	27.500	600	Neg.	Neg.	Neg.

Fanasil, 5 were failures. Out of 14 treated with Fanasil + pyrimethamine 2 were failures. However, on the average the parasitemia disappeared as quickly as in *falciparum* cases that responded. Higher doses should be tried in view of the fact that another investigator in Indonesia has obtained preliminary results in *P. vivax* which are at least as good as those he obtained in *P. falciparum* (61).

c) *P. malariae and P. ovale*. The scanty material available on these two species seems to indicate that they respond worse to Fanasil than *P. falciparum*, but that the association Fanasil + pyrimethamine is active.

Suppressive treatment with Fanasil alone and combined with pyrimethamine.

a) *P. falciparum*. The effect of single and weekly administration of Fanasil, pyrimethamine, and their combination on parasitemia due to *P. falciparum* in schoolchildren was extensively studied by Laing in Tanzania (66, 72). The results are summarized in Table XI. It seems that in schoolchildren doses as low as 125 mg Fanasil weekly were capable of suppressing asymptomatic asexual parasitemia in the great majority but not in all cases. No failures were seen when this dose (or even less) was combined with 6.25 or 12.5 mg pyrimethamine. Pyrimethamine alone was not effective since 38% and 18% of the children still had asexual parasitemia 7 days after one dose of 25 and 75 mg respectively. Even after 4 to 6 weekly doses of 25 mg pyrimethamine, 27% of the children were still positive.

In the same holoendemic area in Northeastern Tanzania, Pringle and Lane (89) had the opportunity of testing the suppressive effect of Fanasil and Dapsone on break-through parasitemias among children previously injected with one of the above mentioned repository antimalarial agents. These substances had been administered during a drug trial conducted 5 months previously. As shown in Table XII a single dose of 100 mg Fanasil was first given to patients of the biguanide and sulfone groups. Out of 34 trophozoite carriers, only two had scanty parasitemia one week later, whereas 13 were again positive after one month. At that time the patients of these two groups received one 100 mg dose of Dapsone which failed to suppress parasitemia in 2 cases. Two and a half months later, the three groups (biguanide, sulfone and combined) were given either 100 mg Fanasil (68 patients) or 100 mg Dapsone (54 patients). Fifty days later the same patients received 50 mg Fanasil or Dapsone. From the overall results it appears that Fanasil was the more effective drug in suppressing such break-through parasitemias.

Shute and Dowling (110) compared the suppressive action of Fanasil and chloroquine on parasitemia in schoolchildren living in the Western region of Nigeria where malaria is holoendemic. As shown in Table XIII, the children of each of three large schools received one of the following drug regimens: Fanasil 500 mg once a week for 4 weeks, Fanasil 500 mg as a single dose, or chloroquine 300 mg as a single dose. No treatment was given in two smaller schools where the children were considered as controls. Blood surveys (thick blood films) were carried out before the beginning of the trials and at weekly intervals for 4 weeks thereafter.

Table XI

Effect of Fanasil and pyrimethamine given alone or in combination against pyrimethamine-resistant Plasmodium falciparum in semi-immune schoolchildren with a pre-treatment parasite incidence of over 25% (Laing, Tanzania[66,72])

No. of children examined	Dosage	No. of children with trophozoites
Fanasil alone		
176	500 mg/week for 4-6 weeks	2
140	250 mg/week for 8 weeks	1 (after 2 doses) 0 (after 4 doses) 2 (after 8 doses)
126	125 mg x 1	0 (1 P. ovale)
37	100 mg x 1	4
36	75 mg x 1	1
35	50 mg x 1	2
33	25 mg x 1	3
Fanasil (F) + pyrimethamine (P)		
178	F 500 mg + P 25 mg once weekly for 4-6 weeks	0
159	F 250 mg + P 12.5 mg/week for 8 weeks	0 (after 2 doses) 1 (after 4 doses) 0 (after 6 & 8 d.)
125	F 125 mg + P 12.5 mg x 1	0
54	F 100 mg + P 12.5 mg x 1	0
83	F 75 mg + P 12.5 mg x 1	0
39	F 50 mg + P 12.5 mg x 1	2
37	F 25 mg + P 12.5 mg x 1	4
39	F 125 mg + P 6.25 mg x 1	0
29	F 100 mg + P 6.25 mg x 1	0
33	F 75 mg + P 6.25 mg x 1	1
Pyrimethamine alone		
133	12.5 mg for 8 weeks	17 (after 2 doses) 19 (after 4 doses) 17 (after 6 doses) 16 (after 8 doses)
122	75 mg x 1	22 (18%)
123	25 mg x 1	47 (38%)
684	25 mg/week for 4-6 weeks	184 (27%)
Untreated		
67		48 (71%)

Response of asymptomatic parasitemias occurring among children (previously injected with a repository antimalarial) to single doses of Fanasil or Dapsone

(Pringle and Lane, Tanzania[89])

Drug and dose	Type of repository drug	Days since injection	No. of patients	Patients with trophozoites of P. falciparum before and after the "super-treatment"	
				Before treatment	One week later
Fanasil 100 mg	Biguanide	150	57	16	1 *
	Sulfone		53	18	1 *
Dapsone 100 mg	Biguanide	182	59	6	0
	Sulfone		59	7	2
Fanasil 100 mg	Biguanide	254	28	13	0
	Sulfone		22	10	0
	Combined		18	11	0
Dapsone 100 mg	Biguanide	254	13	6	1
	Sulfone		21	8	3
	Combined		20	6	0
Fanasil 50 mg	Biguanide	305	29	13	0
	Sulfone		22	10	0
	Combined		18	11	0
Dapsone 50 mg	Biguanide	305	13	6	0
	Sulfone		24	10	1
	Combined		19	10	0

* in both of these cases the parasite density was extremely low

The untreated controls showed continuing *falciparum* parasitemia in 69 to 81% of the cases throughout the trial. Both regimens of Fanasil were found to be highly effective against asexual *P. falciparum* parasitemia as from the first week when all cases were negative. In the Fanasil groups, the highest incidence of children with trophozoites was 2% after 4 weeks (in the single dose group), whereas in the chloroquine group the incidence of positive cases was 5% after one week and progressively increasing to 16% after 4 weeks.

b) *P. malariae*. Shute and Dowling (110) also registered quite a high pre-treatment incidence of asexual parasitemia due to *P. malariae* during the surveys in the above mentioned Nigerian schools. The rates of positive cases before and after treatment are given on the extreme right of Table XIII which shows a rate of 10 to 15% prior to treatment, increasing to 19% after 4 weeks in the control group. This table also shows that the effect of chloroquine on *P. malariae* was entirely satisfactory and that both regimens of Fanasil were almost as effective as chloroquine.

Recent (unpublished) work carried out in Indonesia (61) indicates that Fanasil given alone or together with pyrimethamine is capable of completely suppressing *P. malariae* parasitemia by the second week after the first dose. Confirmatory trials are being undertaken.

c) *P. vivax and P. ovale*. The number of cases treated with Fanasil is too small to permit evaluation of the results. In Africa, Laing (72) observed the emergence of P. *ovale* in 2 patients having received weekly doses of 250 mg Fanasil. In another case trophozoites persisted after one dose of 1 g Fanasil. Shute and Dowling (110) observed persistence of *P. ovale* in 6 schoolchildren treated with Fanasil. In 3 further cases the trophozoites apparently emerged during suppressive treatment with Fanasil. In Indonesia, clearance of *P. vivax* parasitemia seems to have been rather slow, complete clearance having been achieved 3 weeks after the beginning of treatment with Fanasil, with or without pyrimethamine, given as a single dose or once weekly.

Gametocytocidal effect: According to the findings reported by Shute and Dowling (110) and by Laing (66, 72), Fanasil has actually no gametocytocidal effects, the slow disapearance of sexual forms of *P. falciparum* being the result of the schizonticidal action of the drug.

Effect on sporogony: Investigations were carried out by Laing (70, 72) to determine the effect of Fanasil alone and combined with pyrimethamine on *falciparum* sporogony. Batches of laboratory-bred *Anopheles gambiae* were fed on patients with sexual parasitemia after treament. All surviving mosquitoes were negative after having fed on 2 patients previously treated with 500 mg Fanasil + 12.5 mg pyrimethamine. The same result was obtained in a further 2 patients after the combination of 500 mg Fanasil and 6.25 mg pyrimethamine. However, when a 1 g dose of Fanasil alone was given to a patient on two occasions, a significant number of mosquitoes were found to be positive after each dose. Consequently Fanasil alone does not seem to affect normal *falciparum* sporogony in mosquitoes. When given together with a small dose of pyrimethamine, which may be insufficient in itself, there appears to be potentiation of the sporontocidal effect of the latter. In experimental malaria (*P. gallinaceum*) such a potentiation has already been described by Ramakrishnan et al. (91) for DDS, although given by itself this substance did not show any sporontocidal effect.

Table XIII

Effect of Fanasil and chloroquine on asexual parasitemia in African schoolchildren of 6 to 14 years (control group 6 to 11 years) (Shute and Dowling, Nigeria[110])

Drug and dosage	Blood surveys	No. of patients examined	P. falciparum		P. malariae	
			Positive	Rate %	Positive	Rate %
Fanasil 500 mg once a week for 4 weeks	Before treatment	209	161	77	21	10
	after: 1 week	195	0	-	5	3
	2 weeks	194	0	-	0	-
	3 weeks	189	1	0.5	0	-
	4 weeks	185	1	0.5	0	-
Fanasil 500 mg single dose	Before treatment	261	175	67	39	15
	after: 1 week	240	0	-	2	1
	2 weeks	237	2	1	0	-
	3 weeks	238	1	0.5	1	0.5
	4 weeks	233	4	2	0	-
Chloroquine 300 mg single dose	Before treatment	248	185	75	25	10
	after: 1 week	222	5	2	0	-
	2 weeks	216	8	4	0	-
	3 weeks	223	21	9	0	-
	4 weeks	147	24	16	0	-
Untreated controls	Before trial	78	60	77	10	13
	after: 1 week	89	61	69	11	12
	2 weeks	76	56	74	13	17
	3 weeks	81	62	76	7	9
	4 weeks	84	68	81	16	19

S U M M A R Y

Review of the early literature as well as more recent results show that sulfonamides possess a distinct antimalarial activity. However, when give alone, their action is less marked and slower than that of the antimalarials commonly used in the treatment of the acute attack. Combinations with pyrimethamine provide better results, even in cases of pyrimethamine and chloroquine resistance. This warrants further investigations in an attempt to develop a therapeutic agent suitable for the treatment of such resistant cases. It may also be possible with an appropriate combination of pyrimethamine with a sulfonamide to achieve a satisfactory method for suppressive treatment both in areas with and without pyrimethamine resistance. Three main points must still be carefully studied: 1) the risk of developing malaria resistance against one or both of the components of the combination. 2) The risk of developing bacterial resistance to sulfonamides if these substances are used on a large scale in too low doses. It seems indeed that antimalarial effect with the combination of sufonamides + pyrimethamine can be obtained with doses of sulfonamides which are below those usually employed in bacterial diseases. Since the range of the ratios providing potentiation is rather large, only ratios of the combination sulfonamides: pyrimethamine should be chosen in which an antibacterial sulfonamidemia is guaranteed. 3) It goes without saying that, although both pyrimethamine and modern sulfonamides, when given by themselves, have proved to possess a large margin of safety, long term administration of their combination should be careful studied from the point of view of possible side effects.

Substantial evidence has already been produced to show that the long acting sulfonamide Fanasil (Ro 4-4393) given once or once weekly possesses marked schizonticidal activity against P. falciparum. *Although its action is slower than that of 4-aminoquinolines, it may be useful as a second choice drug in semi-immune subjects for the therapy of* falciparum *malaria. Preliminary results show that, when combined with pyrimethamine, Fanasil is highly effective in suppressing fever and asexual parasitemia due to* P. falciparum. *Single doses of 1 g Fanasil together with 50 mg pyrimethamine seem to be adequate for the treatment of acute* falciparum *malaria in semi-immune patients. The onset of action of the combination is much more rapid than that of the single components. Weekly doses of 500 mg Fanasil and 25 mg pyrimethamine appear to provide satisfactory suppressive effects against* P. falciparum *at least in East Africa. This combination is active on strains which do not respond satisfactorily to the standard doses of pyrimethamine and/or chloroquine and seems to have a satisfactory sporontocidal effect.*

Preliminary results indicate that Fanasil alone *cannot be recommended for use against the other human malaria parasites. The combination with pyrimethamine appears to be much more effective. East African strains of* P. malariae *seem to respond better to the combination than do Malayan strains of* P. vivax *but further trials are required before definite assessment can be made.*

Fanasil by itself has no gametocytocidal or sporontocidal action but seems to potentiate the effect of pyrimethamine at least on sporogony of P. falciparum.

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