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THE ACTION OF ANTIMALARIAL DRUGS IN MICE INFECTED WITH *PLASMODIUM BERGHEI*

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This paper describes experiments in which several known antimalarial drugs (quinine, proguanil, pamaquin, mepacrine, chloroquin, sulphadiazine, sulphanilamide, and 4 : 4′-diaminodiphenyl sulphone) were tested for activity against infections of *Plasmodium berghei* in mice, in order to assess the possible use of this newly discovered malaria parasite in chemotherapeutic screening.

P. berghei is a parasite of a wild rodent in the Belgian Congo and was discovered and described by Vincke and Lips (1948). It is readily transmitted to white mice by syringe-passage; in experimental infections it produces a parasitaemia which is detectable in stained blood films from the day after inoculation, and which increases steadily until 50–80 per cent of the erythrocytes are parasitized; the mice die in 8–18 days after infection. No mosquito is known which transmits *P. berghei* in the laboratory, and, as yet, no exoerythrocytic form has been described.

Several avian malaria parasites are maintained in the laboratory, and *P. gallinaceum* is now used widely for routine chemotherapy tests in chicks. However, the pharmacology and toxicology of drugs show differences between birds and mammals, and, therefore, a test on a parasite of mammals might give results more readily applicable to human malaria. *P. berghei* is the only plasmodium known which will readily infect the smaller laboratory mammals.

METHODS

The strain of *P. berghei* used in these investigations was received from Dr. P. Brutsaert through Professor H. E. Shortt. We wish to thank the Belgian workers for kindly making the infection available for study.

The method of testing drugs on *P. berghei* infections was based upon the standard tests with *P. gallinaceum* given in the American "Survey of Antimalarial Drugs, 1941–1945," and described by Curd, Davey, and Rose (1945) and Tonkin and Hawking (1947).

Mice, ranging in weight from 15–20 g., were infected for the tests with an inoculum containing 5–15 million parasites in 0.2 ml. citrated blood, injected by the intraperitoneal route. The doses of drugs were adjusted to the weight of the mouse, but all mice received the same inoculum. Drugs were given orally once daily for four days, commencing three to four hours after inoculation. Thin blood films, stained with Giemsa, were examined on the fifth day (i.e., the day after the last dose of drug was administered) and the seventh day. The degree of infection was recorded as the percentage of erythrocytes which were parasitized, and the geometric mean was taken for each group of mice. The Minimum Effective Dose (M.E.D.) was defined as the

smallest dose which gave a mean infection level of less than one per cent of the erythrocytes parasitized on the fifth day. No attempt was made to record the actual percentage of erythrocytes parasitized when the figure was less than one per cent, as this level of infection was taken as the criterion for the M.E.D. Such infections were shown as $<$ 1 per cent, whether the number of parasitized erythrocytes was 1 in 400 erythrocytes or 1 in 20 microscope fields. Twenty oil-immersion fields were examined before a blood film was considered negative.

Mice in which the infection had responded to treatment were examined twice weekly after the seventh day until parasites were again observed in the blood films, or until the sixtieth day. In case there was some immunity reaction which prevented the multiplication of *P. berghei*, mice that had negative blood films up to the sixtieth day were then killed and the blood injected into normal mice. If none of the latter became infected it was assumed that the original mouse had been cured, that is, that the infection had been completely prevented.

The following salts of the drugs were used: quinine hydrochloride containing 88.7 per cent anhydrous quinine, proguanil acetate containing 69.3 per cent of proguanil base, pamaquin methylene bis-hydroxynaphthoate containing 45 per cent of base, mepacrine hydrochloride containing 91.5 per cent of mepacrine base, chloroquin diphosphate containing 62.6 per cent of chloroquin base, sodium sulphadiazine containing 91.5 per cent of sulphadiazine. All amounts of the drugs quoted refer to these salts. Sulphanilamide and 4 : 4′-diaminophenyl sulphone were used as the pure substances. The latter compound is the parent substance from which promin was derived. It is believed that the activity of promin is due to the slow liberation of the parent substance in the body.

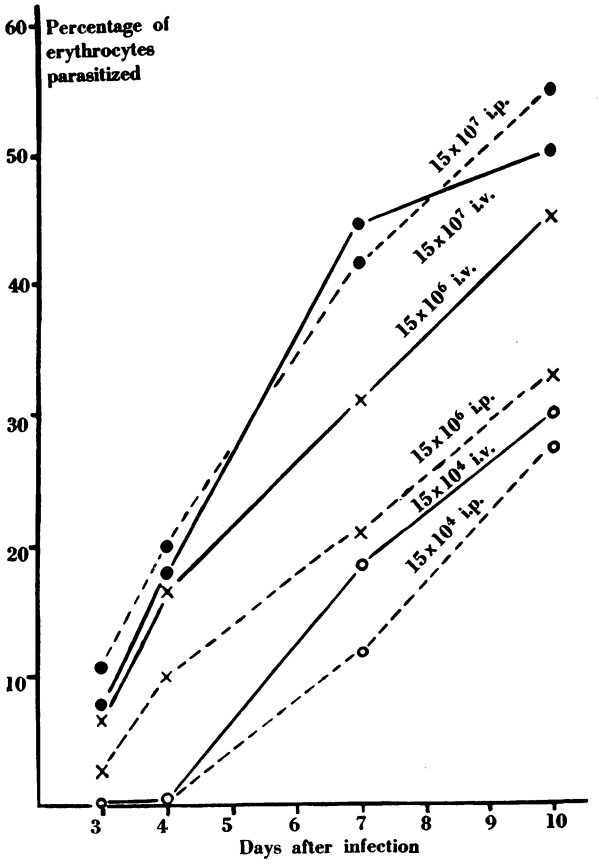

FIG. 1.—To show the course of *P. berghei* infections in mice when different numbers of parasites are injected intravenously or intraperitoneally.

RESULTS

Size of inoculum and route of injection

In preliminary experiments, mice were inoculated with different numbers of parasites by the intravenous and by the intraperitoneal routes, respectively. Table I shows that, on the fifth day, although there was little difference in the degree of infection between the

TABLE I

SHOWING THE MEAN PERCENTAGE OF ERYTHROCYTES PARASITIZED AFTER INTRAVENOUS AND INTRAPERITONEAL INOCULATION, RESPECTIVELY

Mice shown in experiment 1 were examined on the 4th day after infection; those in experiments 2 and 3, on the 5th day

No. of parasites injected per mouse	Mean percentage of erythrocytes parasitized					
	Experiment 1		Experiment 2		Experiment 3	
	i.v.	i.p.	i.v.	i.p.	i.v.	i.p.
150 million ..	18	20				
50 million			25.4	24.5		
15 million	16.5	10				
10 million					31	41
5 million			17.8	7.4		
1.5 million	6.3	1.8				
1 million					13.1	13.4
500,000			< 1	< 1		
150,000	< 1	< 1				
100,000					1.8	0
50,000			0	0		

mice infected by these two routes, there was considerable variation between one experiment and another. The course of the infection in one series of experiments is given in Fig. 1 which shows that the degree of infection obtained with *P. berghei* is not closely related to the number of parasites injected. Accordingly, in framing the tests, the intraperitoneal route was chosen for the injection of the parasites, as being quick and easy to perform. The inoculum of 5–15 million parasites was chosen because it gave a high infection on the fifth day (the mean number of erythrocytes parasitized in six experiments being 20.7 per cent), and mice die in 8–13 days ; also, with this size of inoculum it is possible to obtain from a heavily infected mouse sufficient parasites to infect a large number of mice.

The activity of antimalarial drugs

The results obtained by testing drugs according to the above method are shown in Table II. At least three mice were used for each dose, and the mean results are recorded. The results of several experiments are shown for each drug, together with the figures for the control mice in each experiment. When a dose cleared the blood of parasites, the number of days after inoculation that the blood remained parasite-free is recorded. With quinine and proguanil, cures were rare even at the toxic limits, whereas mepacrine and sulphadiazine readily produced complete cures.

DISCUSSION

Comparison of P. berghei *with* P. gallinaceum

Tests of several antimalarial drugs against *P. berghei* have been reported by Goodwin (1949) and by Schneider, Decourt, and Montézin (1949) and their figures for the Quinine Equivalents can be compared with those obtained with *P. gallinaceum*. The Quinine Equivalent of an antimalarial drug, as defined by Marshall (1946) in the " Survey of Antimalarial Drugs," is the ratio by weight of the dose of quinine

TABLE II

THE ACTIVITY OF ANTIMALARIAL DRUGS AGAINST *P. berghei* INFECTIONS IN MICE, DOSES BEING GIVEN ORALLY ON FOUR SUCCESSIVE DAYS

Drug	Dose mg./20 g.	Mean percentage of erythrocytes parasitized on 5/6th day				Days mice remained negative	Minimum effective dose mg. per 20 g.
		a	b	c	d		
Quinine hydrochloride ..	1.0	9.8		5.8			
	2.0	1.9		2			3.0
	3.0			< 1			
	4.0		0	< 1		2 mice, 13	
	6.0		0	0			
	10.0		0			1 mouse, 21; 1 cured	
Controls ..		13	35	16.3			
Proguanil acetate	0.2	8	19.7				
	0.3		2				0.5
	0.4		< 1				
	0.5	0	1.1	0		2 mice, 13; 1 cured	
	0.7			0		1 mouse, 14	
	1.0			(LD 75) Toxic			
Controls ..		17	37.8	35			
Pamaquin naphthoate ..	0.03	16.8					
	0.1		26		8.6		
	0.2		22.6		8		0.7
	0.5	0	12.2	0	0	6 mice, 9	
	0.7			0	< 1	2 mice, 11; 1 cured	
	1.0	0		0		3 mice, 11; 3 cured	
	2.0	0				3 mice, 13	
Controls ..		17	20	17.4	34.4		
Mepacrine hydrochloride ..	0.05	14	39.1				
	0.1		50				
	0.2		27.1				0.4
	0.4		0			1 mouse, 16; 2 cured	
	0.6		0			3 mice cured	
	1.0	0	0			6 mice cured	
Controls ..		17	37.8				
Chloroquin diphosphate ..	0.0016	17.8					
	0.016	14					
	0.064	15.3	14.1				0.16
	0.096		7.1		1.4		
	0.128	0					
	0.16		2	0	< 1	3 mice, 7	
	0.32			0	0	4 mice, 12	
	0.8			0		1 mouse, 12; 2 cured	
Controls ..		18.4	20	17.4	34.4		

TABLE II—*continued.*

Drug	Dose mg./20 g.	Mean percentage of erythrocytes parasitized on 5/6th day				Days mice remained negative	Minimum effective dose mg. per 20 g.
		a	b	c	d		
Sodium sulphadiazine ..	0.0005		24.1				
	0.001		24.1	15.5			
	0.002			3.9			0.004
	0.004			0		2 mice, 10; 1 cured	
	0.007			0		3 mice, 9	
	0.01		0	0		1 mouse, 10; 1 cured	
	0.1	0	0			1 mouse, 15; 2 cured	
	1.0	0					
	4.0	0					
	10.0	0					
	20.0	0				4 mice cured	
Controls ..		19.8	20	17.4			
Sulphanilamide..	0.001	13.1					
	0.01	11.9					
	0.1	6.2					1.0
	1.0	< 1					
	10.0	0				3 mice, 10	
Controls ..		16.3					
4:4′-Diaminodiphenyl sulphone ..	0.001	< 1					
	0.01	0				3 mice, 9	
	0.1	0				3 mice, 10	0.001
	1.0	0					
	10.0	0					
Controls ..		16.3					

to the dose of the drug under assay when both drugs, administered under identical conditions, produce the same response in parasitized animals. In the present series of experiments this has been taken as the ratio:

$$\frac{\text{Minimum Effective Dose of quinine}}{\text{Minimum Effective Dose of the drug under assay}}$$

calculated as base in each case. In Table III the Quinine Equivalents obtained with *P. berghei* in this laboratory are compared with the results reported by Goodwin, and with the figures obtained when drugs are tested against *P. gallinaceum*. The figures given for the M.E.D. of quinine and the Quinine Equivalents of the other drugs when tested against *P. gallinaceum* are taken from the " Survey of Antimalarial Drugs, 1941–1945," with the exception of the figures for proguanil, which are taken from Davey (1946). The Quinine Equivalents reported by Goodwin (1949) have been recalculated on the amount of base in each drug. They show fair agreement with those found in this laboratory for proguanil and mepacrine, and less close agreement for pamaquin and chloroquin.

TABLE III

QUININE EQUIVALENTS OF VARIOUS ANTIMALARIAL DRUGS TESTED AGAINST *P. berghei* AND *P. gallinaceum* INFECTIONS

Drug	Quinine equivalents		
	P. berghei (mice)		*P. gallinaceum* (chicks)
	Thurston	Goodwin	
Proguanil	7.7	6.6	8
Pamaquin	8.5	4.5	10–40
Mepacrine	7.3	8.5	2–6
Chloroquin	26 6	13	15
Sulphadiazine	727		0.8
Sulphanilamide	2.5		0.08
Promin	—		0.04
4: 4′-Diaminodiphenyl sulphone	2,500		—
Quinine	1 M.E.D. 2.7 mg./20 g. daily×4	1	1 M.E.D. 0.6 mg./20 g. b.i.d.×8

P. berghei is only half as sensitive to the action of quinine as *P. gallinaceum*; similarly, it is also less sensitive to the other antimalarials, with the important exception of the sulphonamides. This difference in sensitivity, however, is not large enough to prevent *P. berghei* from being used in the search for new antimalarials. The Quinine Equivalents show that *P. berghei* is relatively less sensitive to pamaquin than *P. gallacineum* is; the sensitivity to proguanil is almost the same in both species; *P. berghei* is slightly more sensitive than *P. gallinaceum* to mepacrine and chloroquin, while it is many times more sensitive to the action of the sulphonamides.

The results obtained by Schneider, Decourt, and Montézin (1949) have not been included in Table III as the drugs were administered subcutaneously and so cannot be compared with the other figures for *P. berghei* and *P. gallinaceum*. They found that *P. berghei* was two and a half times as sensitive to pamaquin as to quinine, two to four times as sensitive to mepacrine as to quinine, and four to eight times as sensitive to chloroquin (resorchin) as to quinine.

The sensitivity of malaria parasites to sulphonamides

Malaria parasites vary considerably in their sensitivity to the action of sulphonamides. Of the avian species, *P. gallinaceum* is the most sensitive, with a Quinine Equivalent for sulphadiazine of 0.8, while *P. lophurae* and *P. cathemerium* are only slightly sensitive (Survey of Antimalarial Drugs, 1941–1945). *P. relictum* var. matutinum, *P. circumflexum*, and *P. nucleophilum* are unaffected by sulphanilamide (Counts and Coulston, 1941).

The malaria parasites of monkey and man are sensitive in varying degrees to the action of sulphonamides. Sulphadiazine and promin are invariably more active

than sulphanilamide. *P. cynomolgi, P. inui,* and the human malaria parasites are sensitive to the sulphonamides, and *P. knowlesi* is extremely sensitive (Coggeshall, Maier, and Best, 1941). The Quinine Equivalent of sulphadiazine against *P. knowlesi* is 175 (Richardson *et al.*, 1946).

P. berghei is even more sensitive than *P. knowlesi* to the sulphonamides, the Quinine Equivalent of sulphadiazine being 727. As it is a large, rapidly reproducing parasite its sensitivity is in line with the suggestion put forward by Coggeshall (1940) that the sulphonamides act most readily against species with a rapid rate of metabolism.

Para-aminobenzoic acid antagonizes the antimalarial action of the sulphonamides; thus it antagonizes the action of sulphadiazine against *P. knowlesi* (Richardson *et al.*, 1946), and the action of sulphaguanidine against *P. lophurae* in chicks (Marshall, Litchfield, and White, 1942). When given alone, *p*-aminobenzoic acid showed slight antimalarial activity against the latter infection. In one of my own experiments with *P. berghei*, 1 mg. *p*-aminobenzoic acid per 20 g. mouse, given by mouth twice daily, completely antagonized the action of 0.01 mg. sulphadiazine per 20 g. mouse daily; 10 mg. *p*-aminobenzoic acid per 20 g. mouse alone had no action upon *P. berghei*. These observations confirm the view that the sulphonamides act against malaria parasites and against bacteria in a similar manner, and suggest that some fundamental difference in metabolism affects the susceptibility of the various species of plasmodia to the action of sulphonamides.

The test against P. berghei *in mice*

As *P. berghei* has been shown to be susceptible, like *P. gallinaceum*, to the action of a wide range of antimalarial drugs, it is proposed to use this infection in screening new compounds for antimalarial activity in our laboratory. An infection in mice is much easier to maintain and handle than one in chicks, and less drug is required per experiment. The adoption of *P. berghei* for routine testing should lead to an appreciable saving of time, labour, and drug. As, however, no convenient insect vector is yet known for *P. berghei* and sporozoite infection is therefore impossible, no experiments on the prophylactic action of drugs can be undertaken with this infection. For such experiments the test with *P. gallinaceum* must be retained.

In routine screening experiments it is proposed to inject the drug intraperitoneally instead of orally as there is more likelihood of discerning slight activity in a new compound by the former method.

SUMMARY

1. Mice were infected with *P. berghei* by means of an inoculum of 5–15 million parasites intraperitoneally. They were treated with antimalarial drugs by mouth on four successive days. Stained blood films were examined on the fifth day, and the Minimum Effective Dose of each drug was determined from these results.

2. *P. berghei* is half as sensitive to the action of quinine as *P. gallinaceum*. On the basis of the Quinine Equivalents, *P. berghei* is as sensitive as *P. gallinaceum* to proguanil; it is less sensitive to pamaquin and slightly more sensitive to mepacrine and chloroquin; it is many times more sensitive than *P. gallinaceum* to sulphonamides.

3. In its susceptibility to sulphadiazine, 4 : 4′-diaminodiphenyl sulphone and sulphanilamide, *P. berghei* resembles the malaria parasites of monkey and man more than it does the avian species. *p*-Aminobenzoic acid antagonized the activity of sulphadiazine.

4. It is proposed to adopt the test against *P. berghei* in mice for routine screening experiments in this laboratory in place of the existing test against *P. gallinaceum* in chicks.

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