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THE ACTION OF DYES, ANTIBIOTICS, AND SOME MISCELLANEOUS COMPOUNDS AGAINST *PLASMODIUM BERGHEI*

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After it had been shown that *Plasmodium berghei* was sensitive to a number of antimalarial drugs (Thurston, 1950) its sensitivity to some dyes, antibiotics, and miscellaneous compounds was tested by a similar technique. Mice were infected with a smaller inoculum than the one used in the previous experiments, because it was found that light infections of *P. berghei* were more susceptible to the action of drugs than heavy infections. It has previously been reported that advanced infections of *P. berghei* were more difficult to cure than earlier ones (Baldi and Rocca, 1951), and that light infections of *P. gallinaceum* were more susceptible to the action of drugs than heavy infections, whether the inoculum consisted of parasitized erythrocytes (Davey, 1946) or of sporozoites (Greenberg, Trembley, and Coatney, 1950).

METHODS

Mice, ranging in weight from 15–20 g., were infected with an inoculum containing 1,000,000 parasites in 0.2 ml. citrated blood, injected intraperitoneally. Drugs were usually given intraperitoneally once daily for four days, commencing five to six hours after inoculation. Thin blood films, stained with 5% Giemsa, were examined on the fifth day and on the seventh day.

Untreated controls had an average infection of 1–5% of the erythrocytes parasitized on the fifth day. The minimum effective dose of a drug was taken to be the smallest dose which on the fifth day reduced the mean infection level to one-fiftieth of that of the controls. A compound was said to show slight activity if, when the maximum tolerated dose was given, the parasitaemia was significantly less than that of the controls but yet was not reduced to one-fiftieth of that figure.

The inoculum and resulting infection rate were lower than those used in the preliminary tests of antimalarial drugs against *P. berghei* (Thurston, 1950), because it was found that the same dose of drug showed much greater activity against a low infection than against a high one. For example, 0.2 mg. of mepacrine per 20 g. mouse cleared the blood for two days when the controls had a mean infection level of 2%, but the same dose only reduced the mean parasitaemia to 28% when the controls had a mean infection of 38%. A drug which was only slightly active at the maximum tolerated dose might easily be missed if tested against too severe an infection.

RESULTS

The results of chemotherapeutic tests with some dyes, antibiotics, and miscellaneous compounds are given in Table I. In this table the figures for the activity against *P. gallinaceum* have been taken from Wiselogle (1946) unless other references are given. It should be noted that drugs were administered by mouth to chicks infected with *P. gallinaceum,* whereas they were given intraperitoneally to mice infected with *P. berghei.* The two sets of figures are therefore not completely comparable although they give a good indication of the degree of activity against each infection. The figures for *P. gallinaceum* are quoted from blood inoculation tests and not from sporozoite infection tests. The quinine equivalent is the ratio

$$\frac{\text{Minimum effective dose of quinine}}{\text{Minimum effective dose of drug under assay}}$$

DISCUSSION

Dyes.—Methylene blue was at one time used in the treatment of human malaria although it had only slight specific action on the parasites (Werner, 1914). It was found that the introduction of a basic dialkylamino group into the side chain resulted in greatly increased activity (Schulemann, 1932). Pamaquin and mepacrine were synthesized during further experiments with similar basic side chains.

Mudrow-Reichenow (1951) showed that methylene blue was active against *P. berghei.* During the present experiments it proved to be the most

TABLE I

THE ACTIVITY OF DYES, ANTIBIOTICS, AND MISCELLANEOUS COMPOUNDS TESTED AGAINST *P. BERGHEI*, COMPARED WITH THEIR ACTIVITY AGAINST *P. GALLINACEUM*

Compound	Maximum Tolerated Dose mg./ 20 g. Mouse, i.p.×4	Activity Against *P. berghei* Minimum Effective Dose mg./ 20 g. i.p.×4	Activity Against *P. berghei* Quinine Equivalent	Activity Against *P. gallinaceum* Quinine Equivalent	Chemical Name
Quinine	2·0	1·0	1·0	Minimum effective dose 3·0 mg./100 g. b i d.×4 oral	
Proguanil	0·2 (b.i.d.)	0·2 (b.i.d.)	5·0	8·0 (Davey, 1946)	
Sulphadiazine ..	5·0	0·005	500	0·8	
Dyes:					
Methylene blue	1·0	0·3	3·3	0·06 oral	3 : 7-*bis*(Dimethylamino)phenazathionium chloride
Toluidine blue ..	1·0	0·5	2·0	0·04 diet	3-Amino-7-dimethylamino-2-methyl phenazathionium chloride
Thionin blue ..	2·0	1·0	1·0		3-Dimethylamino-7-ethylmethylamino phenazathionium chloride
Methylene violet (Bernthsen)	2·0	1·0	1·0	0·15 ,,	7-Dimethylamino-3-phenthiazone
Azure A ..		1·0	1·0	0·04 ,,	3-Amino-7-dimethylaminophenazathionium chloride
Methylene green	1·0	Slight activity	<1·0	<0·06 ,,	3 : 7-*bis*(Dimethylamino)-6-nitro-phenazathionium chloride
Nile blue ..	1·0	1·0	1·0	<0·03 oral	5-Amino-9-diethylaminobenzo(a)phenazoxonium hemisulphate
Meldola's blue..	0·3	Slight activity	—	<0·04 diet	9-Dimethylaminobenzo(a)phenazoxonium chloride
Brilliant cresyl blue	5·0	2·0	0·5	<0·03 oral (*P. lophurae*)	7-Amino-3-diethylamino-2-methyl phenazoxonium chloride
Gallamine blue	20	Inactive	—	<0·02 diet	1-Carbamyl-7-dimethylamino-3 : 4-dihydroxy phenazoxonium chloride
Thionin ..	5·0	Slight activity	<0·2	0·04 diet	3 : 7-Diaminophenazathionium chloride
Neutral red ..	2·0	Inactive	—	<0·3 oral	3-Amino-7-dimethylamino-2-methylphenazine chloride
Methylene violet (R.R.A.)	0·3	Slight activity	—	2·0 diet	3 : 7*bis*(Dimethylamino).5-phenyl phenazine chloride
Rhodamine B ..	2·0	,, ,,	<0·5	<0·03 oral (*P. lophurae*)	9(*o*-Carboxy phenyl)-3 : 6-*bis* (diethylamino)-xanthylium chloride
Gentian violet ..	0·2	Inactive	—	<0·4 diet	Hexamethyl *p*-rosaniline hydrochloride
Antibiotics:					
Penicillin ..	>10,000 units i.p. >100,000 units oral	Inactive	—	Inactive	
Chloromycetin..	>10	Inactive	—	Inactive at 10 mg./ 100 g. (this experiment)	
Streptomycin ..	>10	,,	—	Inactive (*P. lophurae*)	
Aureomycin ..	2·0	0·5	2·0	0·25 (Coatney *et al.*, 1949)	
Terramycin ..	3·0	1·0	1·0	Inactive at 10 mg./ 100 g. (this experiment)	
Miscellaneous compounds:					
Neoarsphenamine	1·0	Slight activity	—	0·03	
Tartar emetic ..	0·5	0·5	2·0	<0·4 oral	
Anthiomaline ..	3·0	1·5	0·67	<0·02 ,,	
Fuadin ..	10	1·5	0·67	Inactive	
Marfanil ..	>30	Slight activity	<0·03	<0·02 oral	
D.2. Polyoxyethylene ether	25	Inactive			
p-Aminosalicylic acid	4·0	,,	—		
p-Chloroaniline	20	,,	—		
p-Bromoaniline	3·0	,,	—		
p-Aminobenzoic acid	>20	,,	—		
Folic acid ..	>10	,,	—		
Methionine ..	>10	,,	—		
Nicotinamide ..	>10	,,	—		

All figures for *P. gallinaceum* have been taken from Wiselogle (1946) unless other references are given.

active of the series of related dyes. In the phenazathionium compounds it would appear that substitution in the amino groups, i.e., increased potential basicity, was accompanied by an increase in activity. In methylene blue both the amino groups carry two methyl radicles while thionin, in which both the amino groups are unsubstituted, had the lowest antimalarial activity. Methylene green resembles methylene blue, but contains a nitro-group in the 6– position ; it had only slight activity against *P. berghei.* Toluidine blue, thionin blue, methylene violet (Bernthsen), and azure A have various radicles on one or both of the amino groups.

The phenazathionium compounds and phenazoxonium compounds were considerably more active against *P. berghei* than against *P. gallinaceum.* The order of activity was not the same, for methylene violet (Bernthsen) was less active than methylene blue against *P. berghei,* whereas it was 10–40 times as active as any other compound against *P. gallinaceum.*

The other dyes which were tested against *P. berghei* had different types of structure ; they were either inactive or had very slight activity. When tested against *P. gallinaceum* they were more active than the dyes of the methylene blue and nile blue series.

Antibiotics.—Levaditi and Vaisman (1950) found that aureomycin was slightly active against *P. berghei* and that penicillin (100,000 units per mouse) and chloromycetin (40 mg. per mouse) were moderately active when the drugs were given by mouth once daily for three days. Streptomycin was inactive. Penicillin was inactive in human malaria, although there was some indication that it potentiated the activity of quinine (Deshmukh, 1947). Aureomycin was slightly active against *P. vivax,* but did not give radical cures (Cooper, Coatney, Imboden, and Jeffery, 1949).

In the present experiments, penicillin, chloromycetin, and streptomycin were inactive against *P. berghei.* Both aureomycin and terramycin were active against *P. berghei,* aureomycin being twice as active as terramycin. Aureomycin was more active against *P. berghei* than against *P. gallinaceum.* Terramycin was inactive against *P. gallinaceum.*

Miscellaneous Compounds.—Arsenicals were at one time used in the treatment of malaria, though with little effect. Neoarsphenamine was inactive against *P. berghei* when given by mouth (Mudrow-Reichenow, 1951). It was active when given subcutaneously, and the activity could then be antagonized by dimercaprol (Black, 1951). In the present experiments, neoarsphenamine showed only very slight activity when given intraperitoneally.

Tartar emetic, anthiomaline, and fuadin (compounds which contain antimony) were more active against *P. berghei* than against *P. gallinaceum.*

Although *P. berghei* is very sensitive to the action of sulphadiazine it was only slightly affected by marfanil. This drug differs from the sulphonamides, which are active antimalarials, in having the amino group separated from the benzene ring by a methylene group, and unlike them its antibacterial activity is not antagonized by *p*-aminobenzoic acid.

The polyoxyethylene ether (D.2), which is active against *Mycobacterium tuberculosis* (Cornforth, Hart, Rees, and Stock, 1951), and *p*-aminosalicylic acid, which also possesses considerable antibacterial activity *in vivo,* were both inactive against *P. berghei.*

p-Chloroaniline and *p*-bromoaniline were possible degradation products of proguanil and its bromo-analogue ; both were inactive.

p-Aminobenzoic acid, folic acid, methionine, and nicotinamide were inactive against *P. berghei.* Galliard and Lapierre (1950) reported that folic acid had slight antimalarial action against *P. berghei.* Marshall, Litchfield, and White (1942) found slight activity in *p*-aminobenzoic acid when tested against *P. gallinaceum.*

These experiments again emphasize the differences between species of malaria parasites in their sensitivity to drug action and the value of testing potential antimalarials against as many species as possible.

Summary

1. Tests for chemotherapeutic activity were performed against light infections of *Plasmodium berghei,* as these were more sensitive than heavy infections.

2. Methylene blue was the most active of a series of related compounds. Aureomycin and terramycin were active against *P. berghei* ; penicillin, chloromycetin, and streptomycin were inactive. Antimonials were more active against *P. berghei* than against *P. gallinaceum* ; neoarsphenamine had very little effect against *P. berghei.* Marfanil was very slightly active against *P. berghei.*

p-Aminosalicylic acid, D.2, *p*-chloroaniline, *p*-bromoaniline, *p*-aminobenzoic acid, folic acid, methionine, and nicotinamide were all inactive.

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