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# THE TECHNIQUE OF TESTING CHEMOTHERAPEUTIC ACTION ON *PLASMODIUM GALLINACEUM*

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During the war *P. gallinaceum* and *P. lophurae* came into general use for testing the antimalarial action of new compounds. The purpose of the present work, carried out during 1943 and 1944, and withheld from publication owing to wartime restrictions, was to compare different methods of dosage in tests with *P. gallinaceum*, and to see how much the answer obtained depended upon the technique employed.

In all tests the compounds were judged by their ability to reduce parasitaemia to a low level; little or no account was taken of the power of drugs to sterilize the birds and thus to produce a radical cure of the infection.

## METHODS

The general plan of the test was based on confidential information received from the American Board for the Co-ordination of Malaria Studies and from Mr. D. G. Davey, of Imperial Chemical Industries, Ltd.

The strain of *P. gallinaceum* used was obtained from the Molteno Institute, Cambridge, and was derived from the original strain introduced into Europe by Brumpt. It was maintained by blood passage in young chickens. The chickens were about 10 days old at the time of inoculation and weighed 60–80 g.; they were infected by the intravenous injection of about $10^7$ parasitized cells per chick. In a typical experiment the percentage of parasitized erythrocytes, resulting from this inoculum, was 1 per cent after 2 days, 6 per cent after 3 days, 30 per cent after 4 days, and 50–90 per cent after 5 days, at which time chickens often died; if they survived, the parasites in the blood became less numerous, but many died with exoerythrocytic forms in the brain two weeks after inoculation. The drugs were given by mouth with a syringe and blunt needle. Since most drugs supplied for testing are very limited in amount it was considered that the administration of drugs in the diet (as described by Marshall *et al.*, 1942) would not be practicable. The blood of the infected chicks was examined on the 3rd and 5th days and the percentage of parasitized erythrocytes on the 5th day was determined.

To test for the prophylactic activity of drugs, the heads and thoraces of batches of mosquitoes known to contain sporozoites in their salivary glands were ground in saline and the resultant suspension centrifuged lightly to throw down the chitinous parts of the insects. The supernatant was drawn off and made up in a mixture of heparinized chick

plasma and saline, containing at least 50 per cent (v/v) plasma, so that 0.2 c.c. contained the equivalent of one infected mosquito. This suspension was used to infect chicks by intravenous inoculation of 0.2 c.c. per chick. Chicks so infected usually exhibited parasites in the peripheral blood 5–7 days after inoculation, and died 6–10 days after infection as a result of massive infection of the endothelial cells of the cerebral capillaries with exoerythrocytic forms. The drugs were administered in the manner described above, dosage being started 2 hours before infection. In both types of experiment the geometrical mean of the responses of the individual birds in a group was taken; this was compared with the geometrical mean of the responses of the group of untreated control chickens.

The following salts of quinine, mepacrine (atabrine), and pamaquin (plasmoquin) were used: quinine bisulphate containing 59 per cent anhydrous quinine, mepacrine methansulphonate (quinacrine soluble, May & Baker) containing 65 per cent of mepacrine base (3rd ADD. B.P. 1932, p. 15) and the methylene bis-hydroxynaphthoate of 6-methoxy-8-δ-diethylamino-α-methylbutyl)-aminoquinoline (pamaquin) containing 45 per cent of base (4th ADD. B.P. 1932, p. 24). All amounts of the drugs quoted refer to these salts. Sulphadiazine powder was used as the pure substance.

## *Measurement of the blood concentration of the drugs*

An attempt was made to measure the levels of quinine, mepacrine, and sulphadiazine in the blood after the different dose schedules employed. In order to facilitate the taking of blood samples, larger chickens were used in these experiments than in the therapeutic experiments.

*Quinine.*—The blood level of quinine at varying periods of time after dosing was measured by a modification of the method of Kelsey and Geiling (1942), kindly devised by Prof. C. Rimington. The estimations were carried out on 0.5 c.c. blood, drawn from the heart, of 3–4 months old chickens weighing about 800–1,000 g. A series of blood samples was taken from each chicken. The oxalated blood sample was pipetted into 3.5 c.c. distilled water and the blood proteins were digested by the addition of 1 c.c. 10 per cent sodium hydroxide and heating in a water bath for 15 min. The samples were then extracted with 25 c.c. of sodium-dried ether containing 5 per cent (v/v) petrol in a 50-c.c. separating-funnel. The ether extract was washed twice with 10 c.c. $N/10$ sodium hydroxide and the quinine extracted with 4 c.c. $N/10$ aqueous sulphuric acid in three successive volumes of 1.5 c.c., 1.5 c.c., and 1 c.c. of acid. The acid extract was then made up to 5 c.c. by the further addition of sulphuric acid and its fluorescence was measured in the Rimington fluorescence comparator (Rimington, 1943). The amount of quinine present in the extracts was ascertained by comparison with a standard curve obtained by measuring the fluorescence observed when known amounts of quinine were added to blood and extracted as above.

*Mepacrine.*—For the estimations of mepacrine the appropriate doses were given to 800–1,000 g. chickens and blood was withdrawn from the heart. Repeated bleedings of the same chickens sometimes led to false readings of the drug level; consequently several birds were placed on the same dose schedule and no bird was bled more than three times. Coagulation of the blood was prevented by potassium oxalate; if the sample had to wait more than 24 hours before examination it was stored at −12° C. The estimations were kindly carried out for us by Major J. Reid of the Royal Army Medical College; he used a modification of Masen's method (1943).

*Sulphadiazine.*—Appropriate doses of sulphadiazine were given to 28-day-old chicks, 0.02 c.c. blood was taken from the leg at intervals, and the concentration of sulphadiazine therein was measured by a modification of the method of Marshall and Cutting (1938). The volume was measured in special micro-pipettes and washed out into 2.5 c.c. of acid

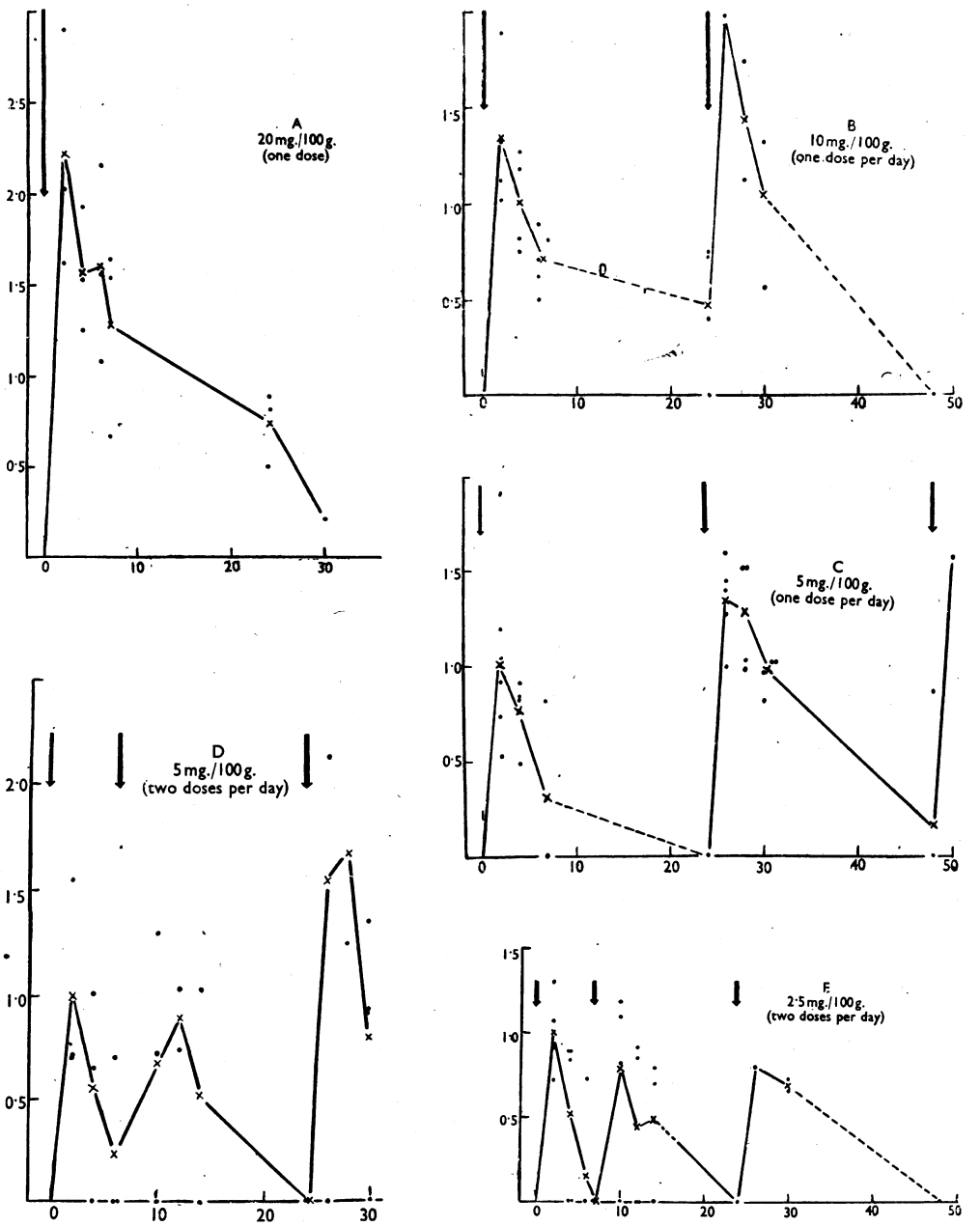


FIG. 1.—Blood concentrations of quinine in chicks after oral administration of different dose schedules of quinine bisulphate. Ordinates: blood concentrations in mg. per litre. Abscissae: hours after dosage. • = levels in individual birds. x——x = average levels. Arrows indicate dosage.

saline (0.85 per cent NaCl in $N/50$ HCl). The reagents, 0.1 c.c. 0.3 per cent sodium nitrite, 0.1 c.c. 1.5 per cent ammonium sulphamate, and 0.1 c.c. 0.1 per cent naphthyl ethylene-diamine dihydrochloride, were then added to 2.5 c.c. of the supernatant, and the intensity of the fully developed colour was measured on a Spekker absorptiometer combined with a spot galvanometer.

## EXPERIMENTS

### TROPHOZOITE-INDUCED INFECTIONS

*Effect of concentration or dispersion of the dose*

The first investigations were made to determine the effect of concentrating the whole amount of antimalarial compound (quinine, mepacrine, pamaquin, or sulphadiazine) into a single administration or of dispersing it over several days in a series of smaller doses. The total amount administered remained the same, an amount being employed which was about the minimum effective level when given according to the most effective regime. A study was made of the results of the different dose schedules on (*A*) the resultant blood-concentration, (*B*) the therapeutic response.

*A—Blood concentrations*

*Quinine.*—Fig. 1 shows the mean curve for the group of birds receiving quinine; it was obtained by taking the average of the blood concentrations at each particular time. Considerable individual variation in the levels was observed in different chickens, particularly in the series 5 mg. twice daily for 2 days and 2.5 mg. twice daily for 2 days. The mean curve was only an approximate indication of what might happen in any special instance. On the whole the levels were comparable with those found by Kelsey *et al.* (1943). The absorption of quinine was rapid, the peak in the blood concentration occurring at approximately 2 hours, but the compound soon disappeared from the blood again, so that the period of antimalarial action was presumably brief in most cases. The level attained in the blood was not directly proportional to the size of the dose, since a dose of 2.5 mg. produced a peak mean blood concentration of 1.0 mg. per litre (Fig. 1E), while a dose of 20 mg. (8 times as great) produced a peak of 2.1 mg., only twice as high (Fig. 1A).

*Mepacrine.*—The blood concentrations of mepacrine are shown in Fig. 2, the line being drawn through the mean concentrations at the different periods of sampling. The variations between the different birds were greater than those between the different dose schedules so that from this small number of birds no reliable conclusion could be drawn. On the whole, it appeared that with mepacrine there was a peak in the blood concentration occurring about two hours after the dose, followed by a prolonged plateau at a lower level. This is well exemplified in the curve obtained after the administration of 8 mg. per 100 g. (Fig. 2A). Marshall and Dearborn (1946a) found that in the treatment of *P. lophurae* infections in ducks by mepacrine the therapeutic response was

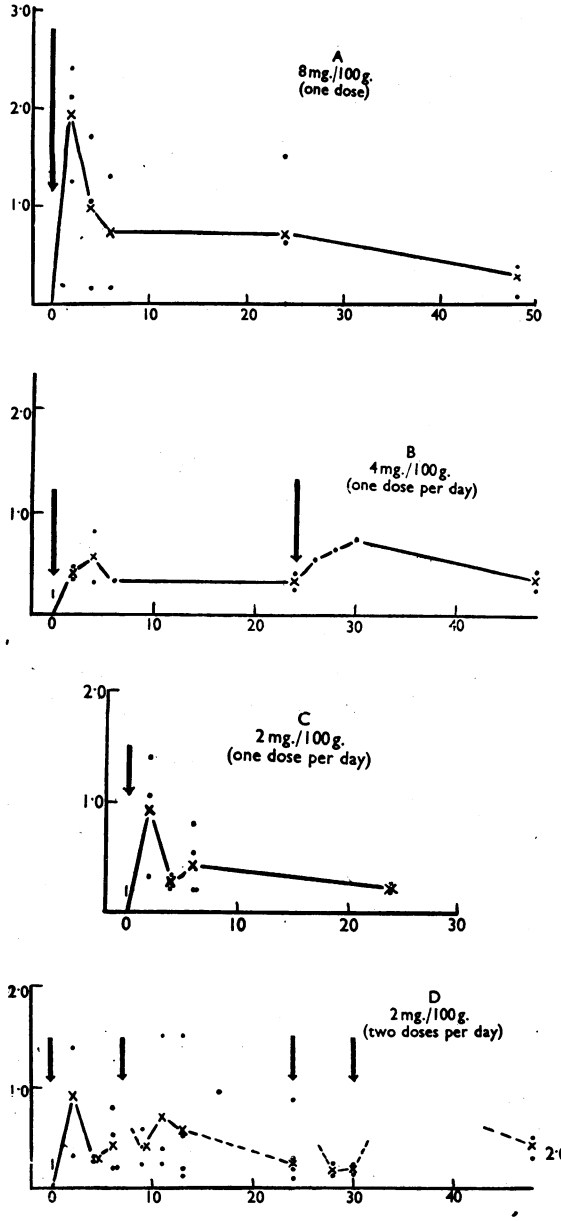


FIG. 2.—Blood concentrations of mepacrine in chicks after oral administration of different dose schedules of mepacrine methanesulphonate. Ordinates: blood concentrations in mg. per litre. Abscissae: hours after dosage. • = levels in individual birds. x——x = average levels. Arrows indicate dosage.

proportional to the dosage rather than to the plasma concentration of the compound. Our own limited figures on blood concentrations and on therapeutic response (Table I) are in agreement with their finding.

*Sulphadiazine.*—The average blood concentrations obtained when sulphadiazine was given are shown in Fig. 3. With one dose per day, even with the largest amount, the drug remained in the blood for less than 24 hours. The mean peak level varied with the dose, but not in direct proportion. After a dose of 25 mg. the peak was 135 mg. per litre; after 200 mg. (8 times the dose) the peak was 254 mg. (Fig. 3A and E). The effect of subdividing the doses was to prolong the periods in which appreciable amounts of drug were present in the blood and to make them more continuous. There was little evidence of an accumulation of sulphadiazine in the blood (Fig. 3E).

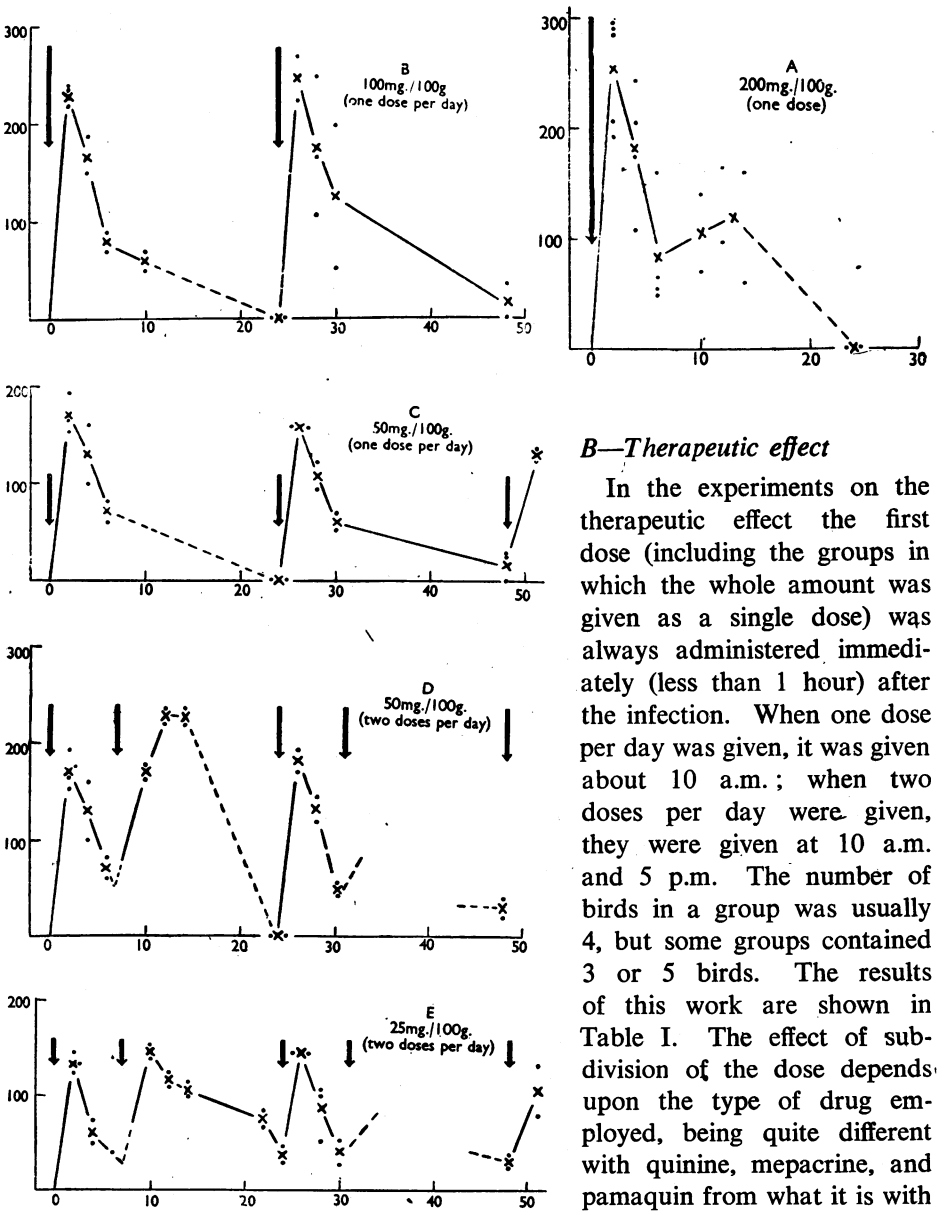


FIG. 3.—Blood concentrations of sulphadiazine in chicks after oral administration of different dose schedules of sulphadiazine powder. Ordinates: blood concentrations in mg. per litre. Abscissae: hours after dosage. • = levels in individual birds. x——x = average levels. Arrows indicate dosage.

### *B—Therapeutic effect*

In the experiments on the therapeutic effect the first dose (including the groups in which the whole amount was given as a single dose) was always administered immediately (less than 1 hour) after the infection. When one dose per day was given, it was given about 10 a.m.; when two doses per day were given, they were given at 10 a.m. and 5 p.m. The number of birds in a group was usually 4, but some groups contained 3 or 5 birds. The results of this work are shown in Table I. The effect of subdivision of the dose depends upon the type of drug employed, being quite different with quinine, mepacrine, and pamaquin from what it is with sulphadiazine. With the former three compounds, the more the dose is concentrated the greater the response, and conversely. The drugs are

TABLE I

THE EFFECT OF DIFFERENT DOSE SCHEDULES OF STANDARD DRUGS ON THE RESPONSE OF TROPHOZOITE-INDUCED INFECTIONS

| Drug | Total dose mg./100 g. | Percentage of cells parasitized on 5th day after different dose schedules | | | | | |
|---|---|---|---|---|---|---|---|
| | | Total dose on 1st day | 1/2 dose once daily 2 days | 1/4 dose twice daily 2 days | 1/4 dose once daily 4 days | 1/8 dose twice daily 4 days | Untreated controls |
| Quinine .. | 20 | 4.1 | 0.52 | 0.59 | 4.7 | 21.6 | 39.3 |
| | | 1.95 | 1.18 | 1.20 | 24.4 | 17.2 | 53 |
| | | — | — | 4.18 | 21.9 | — | 58.2 |
| Mepacrine | 8 | 0.61 | 1.30 | 52.3 | 48.9 | 47.9 | 59.5† |
| | | 0.1 | 0.1 | 1.8 | 3.7 | — | 38.5 |
| | 10 | 0.1 | 0.1 | 0.1 | 1.2 | 3.1 | 65.7 |
| Pamaquin | 0.4 | — | — | 5.24* | 56.7 | — | 54.8 |
| | 0.4 | 0.1 | — | 2.9 | 70.9 | 54.7 | 58.3 |
| | 0.6 | 1.06 | — | — | 3.09 | 9.85 | 44 |
| | | 0.1 | 0.1 | 1.35 | 17.4 | 2.51 | 63.9 |
| | 1 | 0.1 | 0.1 | 0.1 | 0.1 | 1.2 | 41.4 |
| Sulphadiazine .. | 200 | 37 | 28.8 | 38.7 | 12.6 | 0.12 | 39.5 |

* Poor test. Only 1 chick remained alive. † 6th day count.

most effective if given in a single large dose or if the dose is subdivided only into two ; as the subdivisions get more numerous, less antimalarial effect is shown. Apparently the action of these drugs upon the parasites is rapid, a short interval of time being sufficient for it to take place, but on the other hand it is strongly effective only if a certain level of blood concentration is reached—e.g., with quinine, a level of over 1 μg. per c.c. ; this critical level of concentration is presumably not reached when the dose of drug administered falls below a certain quantity, even if the dose is repeated twice daily for four days. With sulphadiazine on the other hand, the greatest antimalarial effect is obtained when the administration is spread over the whole four days, subdividing the amount into 8 small doses. The administration of the drug in 1–4 doses concentrated into the first two days is non-effective. Even the slight modification of giving a single dose on each of four days, instead of two smaller doses on each of these days, greatly diminishes the antimalarial effect. This class of compound presumably exerts a slower action upon the parasites than quinine, mepacrine, or pamaquin do ; and the duration of action is more important than the concentration over any particular short period.

*Effect of time of starting the treatment*

It has been reported from America that some workers begin treatment several hours before infecting the birds, others wait until after the inoculation to begin treatment. The latter procedure is the more convenient in practice as the whole

batch of chicks can be inoculated without the necessity of identifying each individual in the process; moreover, birds in which the inoculation has been unsatisfactory in any way can easily be discarded and replaced by new ones. An investigation was made in order to discover whether this difference of procedure affected the therapeutic response observed, and the results are shown in Table II. As will be seen, there was no significant difference in the response whether the first dose was given immediately after inoculation, or 5 hours before inoculation. Accordingly, in our routine testing of drugs the first dose is always given almost immediately (less than 1 hour) after inoculation.

TABLE II

THE EFFECT OF THE TIME OF STARTING DOSAGE ON THE RESPONSE OF TROPHOZOITE-INDUCED INFECTIONS

| Drug | Total dose mg./100 g. | Dose given twice daily for 14 days | Percentage of cells parasitized on 5th day when dosage started | | Controls |
|---|---|---|---|---|---|
| | | | immediately after infection | 5 hours before infection | |
| Quinine .. | 20 | 2.5 mg. | 10.7 | 19 | 38.5 |
| | | | 3.02 | 14.8 | 41.4 |
| | | | 29 | 24.9 | 61.1 |
| | 24 | 3 mg. | 6 | 7.3 | 44 |
| Mepacrine .. | 10 | 1.25 mg. | 28.3 | 21.5 | 41.4 |
| | | | 0.96 | 1.16 | 44 |
| | | | 9.01 | 5.52 | 61.1 |
| Pamaquin .. | 0.4 | 0.05 mg. | 45.9 | 39.2 | 38.5 |
| | 0.6 | 0.075 mg. | 21.1 | 28.1 | 44 |
| | | | 7.94 | 12 | 63.9 |
| | 1 | 0.125 mg. | 1.2 | 4.59 | 41.4 |
| Sulphadiazine | 200 | 25 mg. | 0.42 | 0.8* | 39.5 |

Each of the groups of treated chicks contained 4 birds; the groups of controls usually contained 6–10 birds.

* Started 2 hours before infection in this case as sulphadiazine reaches its peak in the blood 2 hours after oral administration.

A further study of the effect of delay in commencing treatment was made by giving the same total amount of mepacrine either all on the last (3rd) day after inoculation or spread out over the previous days. This is a reverse of the dose schedules described in Table I. The results are shown in Table III. The best therapeutic responses were obtained when the drug is concentrated on to the last two days of the treatment. Probably in this experiment the effect of concentrating the dosage was more important than the time of starting it. (On this occasion mepacrine seems to have been less effective than in the experiments of Table I.)

TABLE III

THE EFFECT OF DELAY IN COMMENCING TREATMENT WITH MEPACRINE

| Drug | Schedule | % cells parasitized on 5th day | Control |
|---|---|---|---|
| Mepacrine | 6 mg. twice daily on the 4th day .. .. | 2.62 | |
| | 3 mg. ,, ,, ,, ,, 3rd and 4th days .. | 0.56 | |
| | 2 mg. ,, ,, ,, ,, 2nd, 3rd, and 4th days .. | 18.7 | 65.7 |
| | 1.5 mg. ,, ,, ,, ,, 1st, 2nd, 3rd, and 4th days | 35.2 | |

In each group the total dose was 12 mg. per 100 g.
Each group contained 4 chicks.

## SPOROZOITE-INDUCED INFECTIONS

Similar investigations about the effect of concentrating or dispersing the treatment were made with sporozoite-induced infections—i.e., the prophylactic action of the compounds upon the pre-endoerythrocytic forms of the parasites was studied instead of the therapeutic action upon the endoerythrocytic ones (trophozoites, schizonts, etc.). At the time of conducting this work the only compounds known to have a prophylactic action were the sulphonamides, and accordingly the investigations were made using sulphadiazine. Two types of experiment were made. In the first, a given amount of sulphadiazine (200 mg. per 100 g.) was either concentrated at the beginning of the infection or dispersed over the first four days; in the second, treatment was more intense (total 800 mg. per 100 g.) and it was given either in the first two days or on the 3rd and 4th days after infection. The results of both these investigations are shown in Table IV.

In the first type of experiment the data show that the more the treatment was spread out over the first four days the more effective it was; treatment restricted to the first day had little or no effect, even if the total dosage was doubled (i.e., total dose 400 mg.). These results with pre-endoerythrocytic stages of the parasite agreed with those obtained concerning the action of sulphadiazine upon the endoerythrocytic forms; they support the view that the antimalarial action of sulphadiazine is of such a type that duration of exposure is more important than intensity.

The second part of these experiments concerns the sensitivity of the different stages of the parasite to sulphadiazine. Treatment restricted to the first day had little or no action; apparently the sporozoites in their original form or in their early development after inoculation were not much affected by the compound during a single day. Treatment given on the 3rd and 4th days was slightly more effective in delaying the development of patent infection than that given on the 1st and 2nd days; but much of this effect was due to the postponement of treatment, and if the interval is measured between the onset of the patent infection and the end of treatment, there was little real difference between the degree of parasitaemia observed in the two groups. However, ultimate survival was much

TABLE IV

EFFECT OF DIFFERENT DOSE SCHEDULES AND OF TIME OF DOSAGE OF SULPHADIAZINE ON SPOROZOITE INFECTIONS

| | Total dose mg./100 g. | Individual doses per 100 g. | Days of treatment | Percentage of cells parasitized on day | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 8 | 9 | 10 | 11 | 12 | 13 | |
| First part of experiment | 200 | *200 mg. once daily | 1st | | 24.6<br>2 D<br>EEF+ | | 23<br>1 D<br>EEF+ | | | |
| | 200 | 100 mg. once daily | 1st, 2nd | | 1.2 | 2 D<br>EEF+ | 49.3<br>2 D | | | |
| | 200 | 50 mg. once daily | 1st, 2nd, 3rd, 4th | | Less than 0.1 | | 1.6 | | 14.1<br>2 D<br>EEF+ | 2 chicks died on 17th day |
| | 200 | *50 mg. twice daily | 1st, 2nd | | Less than 0.1 | | 0.66 | | 14.9 | 3 chicks died on 15th day. EEF+ |
| | 200 | *25 mg. twice daily | 1st, 2nd, 3rd, 4th | | Less than 0.1 | | Less than 0.1 | | | On 15th day: 51.2. 1 D. EEF+. 1 chick died on 16th day. 1 chick survived |
| | Control | *— | — | | 45.5<br>2 D | | 1 D | | | |
| | 200 | *200 mg. | 1st | 10.2 | 1 D | 48<br>2 D<br>EEF+ | 1 D | | | |
| | 200 | 25 mg. twice daily | 1st, 2nd, 3rd, 4th | Less than 0.1 | | Less than 0.1 | 0.4 | | | 2 chicks failed to show parasitaemia. 2 chicks died on 17th day. EEF+ |
| | Control | — | — | 8.03<br>1 D | 1 D<br>EEF+ | 32.2 | 2 D | | | |
| Second part | 800 | 200 mg. twice daily | 1st, 2nd | Less than 0.1 | | 1.2 | | 6.9 | 2 D | 2 chicks died on 14th day. EEF+ |
| | 400 | 200 mg. twice daily | 1st | 4.3 | 1 D<br>EEF+ | 11.6 | 1 D | 1 D | | 1 chick died on 14th day |
| | Control | *— | — | | 45.5<br>2 D | | 1 D | | | |
| | 800 | *200 mg. twice daily | 1st, 2nd | Less than 0.1 | | 3.1 | 1 D<br>EEF+ | | 42.5<br>2 D | |
| | 800 | 200 mg. twice daily | 3rd, 4th | Less than 0.1 | | Less than 0.1 | | | 24.3 | 1 chick died on 17th day. EEF+. 3 chicks survived |
| | Control | — | — | 8.03<br>1 D | 1 D<br>EEF+ | 32.2 | 2 D | | | |

The inoculum used in these experiments contained the approximate equivalent of 1 infected mosquito per chick. The groups marked with an asterisk contained 3 chicks, the others contained 4.
The "1st day" dose was given 2 hours before infection with sporozoites. D, Chick died.
EEF+ Exoerythrocytic forms found in the brain post mortem.

better in the group treated on the 3rd and 4th days. It is noteworthy that (with the possible exception of 2 chicks) sulphadiazine in these doses failed to sterilize any of the birds ; it only delayed the multiplication of the parasites for a shorter or longer period. In this respect its action upon pre-endoerythrocytic forms was indistinguishable from that upon endoerythrocytic forms. The results thus suggest that the antimalarial action of sulphadiazine is exerted both on pre-endoerythrocytic forms of *P. gallinaceum* (cryptozoites, etc.) and on endoerythrocytic forms (trophozoites and schizonts) ; that this action tends to be plasmodiostatic rather than plasmodiocidal ; and that its effectiveness depends upon the duration of exposure rather than upon the intensity.

## Discussion

It has been shown that under the experimental conditions described the maximum effect of a given small quantity of quinine, mepacrine, or pamaquin upon the trophozoites is obtained when the treatment is concentrated into one or two days. The greatest effect of a given quantity of sulphadiazine upon either trophozoites or upon pre-endoerythrocytic forms is obtained when the treatment is spread out over the whole period of four days. With the former drugs, intensity of action seems more important than duration ; with sulphadiazine, duration is more important than intensity. In the treatment of trophozoite-induced infections there is no significant difference in the response observed whether the first dose is given 5 hours before inoculation or immediately after it. The action of sulphadiazine is exerted both on pre-endoerythrocytic forms of *P. gallinaceum* and on endoerythrocytic ones.

A comparison has been made by Marshall and Dearborn (1946b) of the single-daily-dose and of the drug-diet methods of treatment in bird-malaria (*P. lophurae* in ducks) in relation to the therapeutic activity of numerous compounds ; as found above, they showed that the relative activities of the different compounds are considerably affected by the concentration or dispersion of the dosage.

These results may now be considered in relation to the devising of routine tests of the antimalarial action of new compounds. If the compound were given in a single dose (as is customary in experiments with mouse trypanosomiasis) the action of quinine, etc., would be manifested while that of sulphadiazine would probably be missed. The maximum dose which can be tolerated depends upon the toxicity of the compound, and this is usually such that a series of doses spread over four days is much less injurious than a large dose on a single occasion. Accordingly the usually accepted regime of treatment spread over four days has much to recommend it since (1) it provides sufficient duration for the slower acting compounds such as sulphadiazine, (2) it minimizes toxicity and allows larger amounts of the compound to be given, and (3) it is not unduly laborious to administer. No experiments have been made in this work using schedules extending over more than four days ; as a routine measure such schedules would appear

to be more wasteful of labour and of material (which is often scarce) while offering insufficient compensating advantage. The routine tests carried out in this laboratory are therefore based on treatment lasting four days. The general procedure has been described above in the section upon methods. For infections induced by trophozoites the response is read as the percentage of parasitized erythrocytes on the fifth day, and the geometrical mean for the group of birds is compared with that for the group of untreated controls. If a compound is found to be active in the maximum tolerated dose, the dose is reduced in subsequent tests until the lowest dose is found which reduces the percentage of parasitized cells to about one or two. This is considered to be the approximate minimum effective (therapeutic) dose. For infections induced by sporozoites, the chief criteria of the activity of a drug are complete suppression of infection or a delay in the appearance of parasites in the peripheral circulation, and prolongation of the life of the chicken or recovery from the infection. For quantitative determination of the activity, measurement is made of the minimum effective (prophylactic) dose which prevents the percentage of parasitized cells being greater than 1–2 on the 7th to 9th day when the infection has reached a high level in the controls; no compound tested to date in this laboratory has been sufficiently effective for its activity to be expressed as the minimum dose which completely prevents infection. The antimalarial activity, therapeutic or prophylactic, as expressed by these minimum effective doses, is compared with the toxicity as determined by the maximum tolerated dose for mice, when administered orally twice daily for four days. Since the drugs are designed for use in man, the toxicity for mammals is more significant than that for birds, even though the tests for antimalarial potency are carried out in chickens.

## SUMMARY

The methods used in the authors' laboratory for testing the antimalarial action of drugs upon infections of *P. gallinaceum* induced by trophozoites and sporozoites are described.

A given amount of quinine, mepacrine, and pamaquin exerts the maximum effect on trophozoite-induced infections of *P. gallinaceum* if it is concentrated into the first day or first two days of treatment; with these compounds intensity of action is more important than duration. A given amount of sulphadiazine produces the maximum effect upon trophozoite- or sporozoite-induced infections if it is dispersed over all the four days of treatment; with this compound duration of action is more important than intensity. The action of sulphadiazine is exerted both on the pre-endoerythrocytic forms of *P. gallinaceum* (cryptozoites, etc.) and on the endoerythrocytic forms (trophozoites, etc.).

In the test on trophozoite-induced infections there is no significant difference in the response whether the first dose is given immediately after the inoculation or 5 hours before it.

The blood concentration curves of quinine, mepacrine, and sulphadiazine on the different dose schedules were determined. Increasing the dose eight times increases the peak concentration in the blood only about twice.

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