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N^1-3 : 4-DICHLOROPHENYL-N^5-*ISO*PROPYL DIGUANIDE—A DERIVATIVE OF PROGUANIL HIGHLY ACTIVE IN AVIAN MALARIA

BY

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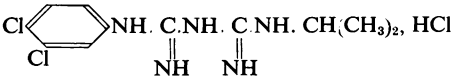
During our study of the antimalarial properties of diguanide substances related to proguanil (" paludrine ") we obtained the impression that weighting the benzene ring augmented activity, and accordingly many compounds were prepared in which disubstitution was made in the benzene ring. Dihalogen substitution (dichloro, dibromo, di-iodo or mixed chloro-bromo, etc.) in positions 3 and 4 proved particularly favourable for antimalarial activity, and a representative compound was selected for trial in human malaria. This substance, given the laboratory number M5943, is N^1-3 : 4-dichlorophenyl-N^5-*iso*propyl diguanide, and has the constitution shown below. It will be noticed that M5943 differs from proguanil only in having an extra Cl atom in position 3 of the benzene ring.



During the last two years trials of M5943 in human malaria have been made in the Liverpool School of Tropical Medicine, the Hospital for Tropical Diseases, London, the Malaria Research Laboratory at Horton (Epsom), and the Calcutta School of Tropical Medicine. It is likely that reports of these trials will appear shortly in the literature, and this account of the laboratory work may therefore be of interest. It is given in more detail than would be possible in the general survey which is being prepared of the antimalarial properties of all the diguanides related to proguanil made in these laboratories.

M5943 is a white, crystalline powder soluble in water to the extent of about 1 g. per 100 ml. It melts at 248–249° C. Solutions may be boiled without decomposition. Its synthesis is described elsewhere (Crowther *et al.*, 1950).

The substance was tested against *Plasmodium gallinaceum* and *P. relictum* (Algerian strain*) by methods described by Davey (1946a, b). Two main types of test were made against each species. One test involves the injection of sporozoites and is designed to demonstrate the action of a drug on exoerythrocytic forms ; the other involves the injection of parasitized red blood cells and is designed to demonstrate its action on the blood forms. Treatment was given orally unless otherwise stated.

* We received this strain through the courtesy of Dr. Ann Bishop.

Activity against P. gallinaceum

(i) The results of the tests against this species are given in Table I, and in Table II they are summarized and compared with results achieved with proguanil in similar tests. It will be seen that M5943 is very much more active than proguanil, in a

TABLE I

ACTIVITY OF M5943 AGAINST THE BLOOD FORMS AND THE EXOERYTHROCYTIC FORMS OF *P. gallinaceum* IN 6-DAY-OLD CHICKS

Activity against exoerythrocytic forms measured after intravenous injection of sporozoites. (Treatment commenced 2–3 hours before the injection of sporozoites; unless otherwise stated it was given orally twice daily for 6 days.)
Activity against blood forms measured after intravenous injection of approximately 50 million parasitized red cells. (Treatment commenced 2–3 hours after infection; it was given orally twice daily for 3½ days.)
Cure = complete prevention of infection. Relapse = marked inhibitory effect on the course of the infection. Delayed death = slight inhibitory effect on the course of the infection.

Activity against exoerythrocytic forms						Activity against blood forms
Dose mg./50 g.	Toxicity	Cures	Relapses	Delayed death	No action	Ratio of mean number of parasitized corpuscles among 500 examined in treated group to mean number in control group. Readings made on the 5th day of infection
0.5 (once daily × 6)	Lethal for many chicks	5	1			
0.4	Lethal for many chicks	15	5			
0.3	Lethal for some chicks	16	2			
0.2		6	4			
0.1		1	11			5.6/379.6 (3 expts., 18 birds)
0.05			13	3		18.3/385.8 (5 expts., 30 birds)
0.025			9	4		55.8/380.5 (5 expts., 30 birds)
0.01			2	11	7	344.6/366.6 (3 expts., 18 birds)

TABLE II

COMPARISON OF M5943 AND PROGUANIL IN *P. gallinaceum* INFECTIONS

Drug	Tested against exoerythrocytic forms		Tested against blood forms
	Protective dose mg./50 g.	Minimum effective dose mg /50 g.	Minimum effective dose mg./50 g.
M5943	0.2–0.3	*circa* 0.025	0.05–0.1
Proguanil	2–3	*circa* 0 25	0.25

weight for weight comparison, against both the blood forms and the exoerythrocytic forms of *P. gallinaceum*.

Unfortunately, the greater activity of M5943, as compared with proguanil, against *P. gallinaceum* is accompanied by a much increased toxicity for chicks. The augmented toxicity has meant that experiments designed to cure completely an established infection are as difficult to execute satisfactorily with M5943 as they were with proguanil (see Davey, 1946b). The earlier experiments with proguanil showed that the longer treatment was delayed after the injection of sporozoites the more difficult it became to cure the infection. In respect of cure, a blood-induced infection behaved immediately like a well-established sporozoite-induced infection. We wondered if cure of established infections would have resulted had we been able to increase the dose of proguanil. The point is of importance in determining if any major difference exists between early and late exoerythrocytic forms, and unfortunately the properties of M5943 have again precluded settling it. As with proguanil, the minimum dose necessary for complete eradication of an early established sporozoite infection is also toxic for some chicks, and by itself it is not sufficient to cure well-established infections. We have tried varying the scheme of treatment in many ways in an effort to use bigger doses of M5943, but all our attempts to cure well-established infections have failed.

(ii) In the general assessment of the antimalarial activity of a substance much importance is attached to the results of a test in which treatment is given intravenously (see Davey, 1946a). The treatment is given on three days only (on Tuesday, Wednesday, and Thursday to chicks infected in the usual way on Monday). In this test the minimum effective dose of M5943, that is the approximate minimal dose keeping the parasite level uniformly low, is 0.1 mg./50 g., which makes it about six times as active as proguanil.

Activity against P. relictum

(i) The results obtained in a test against the parasites of the red blood corpuscles are given in Table III. The minimum effective dose in this test is 0.05–0.1 mg./20 g., which means that M5943 is much more active than proguanil against *P. relictum* also.

TABLE III

ACTIVITY OF M5943 AGAINST *P. relictum*

(Canaries infected intravenously with parasitized blood; oral treatment twice daily × 4½, commencing 2–3 hours after infection; readings quoted as parasites per oil immersion field.)

Dose mg./20 g.	Days of test (infection on day 1)			
	3rd	4th	5th	7th
Controls	1/2–2/1	1/1–10/1	10/1–20/1	2/1–25/1
0.1	1/5–2/1	1/10–1/1	1/25–1/1	0/50–1/5
0.05	1/1–2/1	1/1–4/1	1/10–5/1	0/50–10/1
0.025	2/1	2/1–10/1	5/1–30/1	20/1–30/1

(ii) For the test against exoerythrocytic forms sporozoites were obtained from infected *Culex molestus* and injected intravenously into canaries. Treatment commenced two to three hours afterwards and continued twice daily on each of the next four days. Blood smears were taken from the control birds daily from the sixth day onwards. When they exhibited parasites smears were taken daily from the treated birds. The results are summarized in Table IV. Only the results from birds which survived sufficiently long for cure to be reasonably well assessed (30 days or longer from the time of infection) are included in the Table. Cures were confirmed by subinoculation.

TABLE IV

EFFECT OF M5943 AGAINST A SPOROZOITE-INDUCED INFECTION OF *P. relictum* IN CANARIES

(C = complete protection, R = delay in the appearance of parasites.)

Dose (mg./20 g.)	0.5	0.25	0.1	0.05	0.025
Result ..	5C	6C	5C4R	2C4R	3R

It will be apparent that M5943 has a marked action on the exoerythrocytic forms of this strain of *P. relictum* (proguanil, at maximum tolerated doses, caused no more than a delay before the appearance of parasites in the red blood corpuscles). It will also be apparent that because canaries tolerated M5943 better than chicks we have been able to give them proportionately greater amounts.

(iii) Canaries with a well-established infection of *P. relictum* were treated with M5943, orally twice daily, with doses of varying magnitude. Those receiving 1 mg./20 g. died after four to seven days' treatment, two of three survived 0.75 mg. twice daily for eight days, and three of three survived similar treatment with 0.5 mg. All the survivors, when tested by subinoculation, were shown still to harbour parasites. In other words the better tolerance of canaries for M5943 has allowed us to determine (as we were unable to in chicks) whether doses greater than the minimum giving complete protection against a newly established sporozoite infection would cure an established one, and we have shown that they fail. We still do not know the reason for this.

Toxicity in laboratory animals

Mice and rats.—In mice and rats M5943 is about equally as toxic as proguanil. Figures for the toxicity in mice are given in Table V.

TABLE V

TOXICITY OF M5943 IN MICE

Drug	Approximate LD50 values (mg./kg.)			
	Acute test			Chronic test
	Oral	*i.v.*	*i.p.*	*Oral, twice daily* × 5
M5943	100–150	25	25	25–35
Proguanil ..	50–100	20–25	20–25	25

The toxicity experiments in rats were arranged to show the effect of M5943 on the growth of newly weaned rats. The results were almost identical with those previously obtained with proguanil. Sixty mg./kg. (all doses were given orally once daily) caused deaths; 50 mg./kg. caused an immediate deviation from the normal growth curve and produced scattered deaths; 40 mg./kg. caused a slight deviation from the normal curve and 30 mg./kg. had no apparent effect. Some of the rats receiving 60 mg./kg. and others receiving 50 mg./kg. were killed after they had been treated for 20 days, and subjected to pathological examination by Dr. J. R. M. Innes. Liver, spleen, intestine, kidneys, adrenals, lungs, heart, and pancreas were examined. No macroscopic or microscopic abnormalities were observed.

Chicks.—M5943 is about seven times as toxic to chicks as proguanil when it is given orally, twice daily, for four or five days. A dose of 0.5 mg. to six-day-old 50 g. chicks, administered in this way, kills the majority of chicks 1–5 days after the cessation of treatment. Estimations of the concentration of M5943 in the whole blood of chicks receiving the treatment showed (*a*) that the peak concentration after the first dose rises to just over 1 mg./litre; (*b*) that a residue remains which is built upon for the next three doses until a steady state is reached when the concentration of the residue is of the order of 1.5 mg./litre; and (*c*) that after the ninth dose, peak concentrations superimposed on the residue from the eighth dose reach 3 to 4 mg./litre.

Dogs.—Two dogs were given 10 mg./kg. orally twice daily for five days. One died the day after treatment stopped, the other a day later. Both passed liquid blood-stained faeces on the day before death occurred. A post-mortem examination was made by Dr. J. R. M. Innes and Mr. H. B. Parry, who found that a hyperacute haemorrhagic catarrhal inflammation of the mucosa existed from end to end of the alimentary system except in the fundic end of the stomach and the oesophagus. They examined the rest of the organs macroscopically and microscopically, but considered that the only pathological condition of importance was the acute haemorrhagic gastro-enteritis.

Monkeys.—Several monkeys have been given M5943. Details are as follows:

A female mona monkey, weight 1.6 mg., was given 2 mg./kg. twice daily for two days; then, after a rest of two days, for a further five days, and, after another rest of two days, again for another five days. She gained 200 g. during the experiment and never exhibited symptoms of intoxication of any kind. Three years have passed since the experiment was completed and this monkey is still perfectly fit.

A male mona, weight 1.2 kg., was given 5 mg./kg. once daily for five days, without observable effect. The treatment was then increased to 5 mg./kg. twice daily (12 doses given thus: twice daily $\times 4$, rest $\times 2$, twice daily $\times 2$), then to 10 mg./kg. (20 doses given thus: twice daily $\times 3$, rest $\times 2$, twice daily $\times 5$, rest $\times 2$, twice daily $\times 2$), then to 15 mg./kg. Until the dose of 15 mg./kg. was reached the monkey tolerated the treatment well, but after the second dose of 15 mg./kg. it became obviously ill and treatment was discontinued after the fourth dose. The animal made an uneventful recovery.

A male rhesus, weight 2.3 kg., was given 5 mg./kg. twice daily (70 doses given thus: twice daily $\times 5$, rest $\times 2$, twice daily $\times 5$, rest $\times 2$, etc., for seven weeks). The monkey weighed 2.4 kg. at the end of the experiment. No noticeable symptoms were caused by the treatment.

A female rhesus, weight 2 kg., was given 10 mg./kg. twice daily (30 doses thus: twice daily ×4, rest ×2, twice daily ×5, rest ×3, twice daily ×4, rest ×2, twice daily ×2). The monkey became very quiet and listless during the last few days' treatment, and died two days after the last dose.

Cats.—In its effect on the respiration and blood pressure of cats anaesthetized with chloralose M5943 does not differ significantly from proguanil (Raventós, private communication).

Discussion

From the viewpoint of malarial chemotherapy the most outstanding property of M5943 is its potent action against exoerythrocytic forms. Most authorities agree now that the radical cure and causal prophylaxis of *vivax* (benign tertian) malaria are bound up with the chemotherapy of exoerythrocytic forms, and so it is the success of M5943 in meeting these purposes that will be of most interest. Of course, the very considerable activity of the substance against blood forms may also lead to results of value.

All in all, however, we have to admit we are not optimistic that M5943 will achieve anything not already achieved by proguanil. We are led to this view for two reasons. In the first place the greatly increased antimalarial activity exhibited by M5943 is unfortunately accompanied by a much increased general toxicity. Secondly, the antimalarial action of M5943 is probably the same, fundamentally, as that of proguanil; we know, for example, that a strain of *P. gallinaceum* resistant to proguanil is also resistant to M5943. It would seem, therefore, that M5943 may be fairly described as a more active, more toxic proguanil.

It would, however, be foolish to attempt to forecast too precisely the future for M5943. The parasites of human malaria might be extremely susceptible to it; the human host might have a surprising tolerance for it, or some unusual distribution might give it properties of value. So much could conceivably happen that we asked for M5943 to be tried in human malaria, and tried particularly, at doses as high as possible, in curative experiments against *vivax* malaria. We suggested that the first trials should be made with treatment at 10 mg. per adult (roughly 0.2 mg./kg.) twice daily which is at least one tenth of the amount given to monkeys without observable effect. In arriving at this dose we ignored the fact that M5943 and proguanil are about equally toxic in mice and rats because we have evidence that deaths in these animals after treatment with proguanil are not due to proguanil itself but to a metabolite (Butler, Davey, and Spinks, 1947). It is probable that this metabolite, or something very similar to it, is produced during treatment with M5943, because the magnitude of the toxic doses are similar to those of proguanil and so are the general reactions.

Summary

1. N^1-3,4-dichlorophenyl-N^5-*iso*propyl diguanide hydrochloride, referred to in this report as M5943, is a substance much more active than proguanil (" paludrine," or N^1-4 chlorophenyl-N^5-*iso*propyl diguanide hydrochloride) against both the blood forms and the exoerythrocytic forms of *Plasmodium gallinaceum* and *P. relictum*.

2. In the tests described here 0.05 to 0.1 mg./50 g. twice daily will control a blood infection of *P. gallinaceum* in six-day-old chicks, and 0.05–0.1 mg./20 g. twice daily will control a blood infection of *P. relictum* in canaries.

3. Doses of 0.2–0.3 mg. given twice daily to six-day-old chicks weighing approximately 50 g., will completely protect most of them from infection when sporozoites of *P. gallinaceum* are injected intravenously if treatment commences shortly after infection. Similarly, doses of 0.25 mg. given twice daily to canaries weighing 20 g. will completely protect them from infection with sporozoites of *P. relictum* provided treatment commences sufficiently early.

4. No success was obtained when attempts were made to cure established infections of *P. gallinaceum* and *P. relictum* with M5943.

5. Although the toxicity of M5943 in mice and rats is about the same as that of proguanil, it is more toxic in other animals (chicks, dogs, and monkeys). Monkeys tolerate up to 5 mg./kg. of M5943 given orally twice daily.

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