# ORGANIC ARSENICALS IN THE TREATMENT OF SIMIAN MALARIA

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IT appears from the literature that organic arsenical compounds, such as the salvarsans, have not given results which justify their employment in the general treatment of malaria: a certain measure of success has been reported only in tertian malaria (*P. vivax*). Recently, however, Goldman (1938) has claimed striking results with 'mapharsen' in the same type of malaria: among 24 cases treated with this drug only 2 relapsed, and these were among the group of 14 cases who received but one injection of 0.04 to 0.06 gm. of the drug. On the other hand, Young and McLendon (1939) found that mapharsen failed to eradicate the parasites in

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Calcutta is that one can very rarely be sure of this. Even if one could feel sure, and applied the treatment with either of these preparations, the relapse rate would still be considerable. It is therefore clear that neither of these preparations can be recommended for wide use in the treatment of malarial fever.

It is, however, not impossible that a combined treatment of one of these preparations with quinine might be a very effective treatment for malaria, and that the relapse rate might be considerably reduced. We have no definite evidence on this point and are not at present in a position to study the matter. Nevertheless in *P. vivax* infection, the results of a single injection are usually so dramatic and the fever is so quickly controlled that the writer feels that if he himself developed an attack of malaria due to this parasite, he would feel strongly tempted to start his treatment with one injection of neoarsphenamine, and then to take quinine.

At the same time as the work was being done on human subjects mapharside was supplied to Dr. B. M. Das Gupta for trial in monkey malaria. His findings (reported elsewhere in this journal) are that in *P. knowlesi* infection in monkeys, mapharside has little therapeutic effect.

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REFERENCES

GOLDMAN, D. (1938) .. *Amer. J. Med. Sci.*, **196**, 502.
NIVEN, J. C. (1940) .. Mapharside in the Treatment of Malaria. *Bull. Inst. Med. Res., Federated Malay States*, No. 6. Federated Malay States Govt. Press, Kuala Lumpur.
WERNER, H. (1910) .. *Deut. med. Woch.*, **36**, 1792.

any one of 10 cases of induced quartan malaria (*P. malariæ*), though the symptoms were relieved; the viability of the parasites was not affected. Recently Lowe at the School of Tropical Medicine has been experimenting with arsenicals in the treatment of human malaria, and, as an extension of this work, trials have been made with mapharside (the British name for mapharsen) and novarsenobillon ('N.A.B.') against *P. knowlesi*.

## *Material and methods*

The methods of inducing *P. knowlesi* infection in rhesus monkeys (*S. rhesus*) and of estimating the intensity of the infections have been described elsewhere (Das Gupta and Siddons, 1943). The strain of *P. knowlesi* maintained in this laboratory almost invariably produces typical infections of a progressive and fatal character in monkeys, but the infection can be controlled by an efficient anti-malarial drug. These facts, together with considerations of economy under the present abnormal war-time conditions, have led to the minimum use of control animals in investigations on anti-malarial drugs being conducted in the department.

'N.A.B.' and mapharside were given daily by intravenous injections. The quantity of 'N.A.B.' in an injection varied from 0.0075 gm., or one-twentieth of the human dose of 0.15 gm., for a monkey weighing 1¼ kilos, to 0.1 gm., or one-third of the human dose of 0.30 gm., for a monkey weighing 2 kilos. The dosage of mapharside was 0.004 to 0.01 gm., from one-tenth to one-fourth of the human dose of 0.04 gm., for monkeys weighing 1½ to 3 kilos.

## *Observations with 'N.A.B.'*

The essential data are given under experiments numbered 1 to 6 in the table.

In experiments nos. 1 to 3, quantities of 0.0075 to 0.045 gm. of the drug gave little or no evidence of parasiticidal action, and the infections increased to fatal intensities. The dosage of 0.0045 gm. (experiment no. 3) had some effect on the parasites, for the infection rate of the red cells remained stationary at roughly 6.2 per cent from the fourth to the sixth day of treatment. The parasites then appeared to recover their vitality, and the infection rate rose to 16 per cent, after which the animal was treated with a more efficient anti-malarial drug and the infection was controlled.

With higher doses of 0.06 to 0.09 gm. (experiments nos. 4 and 5), the infections were incompletely controlled and the animals survived; parasites could be found in the peripheral blood for at least eleven days after commencement of treatment. In experiment no. 6, treatment with 0.1 gm. of the drug gave the kind of results expected of an effective anti-malarial agent, with appreciable evidence of direct action on the parasites, but it was not surprising that such a large dose could not be tolerated.

Therefore the administration of 'N.A.B.', while having some action, is not an efficient method of treatment of simian malaria due to *P. knowlesi*.

*Observations with mapharside*

The data for tests with this drug are shown under experiments nos. 7 to 14 in the table.

sporulated, with the result that the infection rate rose from 12.6 to 20 per cent.

In the remainder of the experiments, the infection rate of the red cells was always higher after the course of treatment than before, though usually after the first or second dose a temporary decrease could be noted for one or two

TABLE

*The effect of arsenicals on simian malaria* (P. knowlesi)

| Serial number of experiment | Animal number | Treatment, number of doses × quantity (gm.) | Percentage of infection of R.B.C. | | Remarks |
|---|---|---|---|---|---|
| | | | Before treatment | After treatment | |
| | | | | (*a*) 'N.A.B.' | |
| 1 | 27 | 1 × 0.0075 | 17.0 | ? | Animal died morning after treatment; heart blood showed very heavy infection with normal parasites. Object of experiment was to test effect of drug on morphology of parasites. No effect observed. |
| 2 | 30 | 1 × 0.015<br>2 × 0.03 | 5.8 | 21.4 | Further treatment with mepacrine hydrochloride failed to save the animal. |
| 3 | 32 | 6 × 0.045 | Below 0.1 | 16.0 | Animal had to be treated with a more efficient anti-malarial. |
| 4 | 34 | 7 × 0.06 | 0.8 | 1.4 | During treatment infection rate rose to 9.8 per cent, then decreased to chronic level. Blood became negative only after treatment with another anti-malarial. A small proportion of parasites showed degenerative changes. |
| 5 | 40 | 2 × 0.075<br>5 × 0.09 | 13.2 | Below 0.1 | Parasites persisted for 11 days before further treatment with another drug terminated the infection. |
| 6 | 42 | 2 × 0.1<br>1 × 0.045 | 10.4 | Below 0.1 | Infection reduced to chronic level, but animal died on the 4th day after commencement of treatment—dosage probably not tolerated. |
| | | | | (*b*) MAPHARSIDE | |
| 7 | 44 | 2 × 0.004 | ? | ? | Showed heavy infection when treated; up to 5 per cent of parasites showed degenerative changes after treatment. Animal died. |
| 8 | 49 | 4 × 0.004 | 1.4 | 6.0 | Animal died 2 days after last dose, heart blood showed parasites. |
| 9 | 50 | 5 × 0.004 | 0.4 | 18.2 | Do. |
| 10 | 51 | 1 × 0.008 | 15.2 | 20.0 | Treated when parasites were in the 'ring' stage; 20 hours later mostly normal schizonts present (infection rate 12.6 per cent). These were allowed to sporulate when rate became 20 per cent. Treatment with another drug did not save the animal. |
| 11 | 54 | 3 × 0.008 | 10.0 | ? | Animal died day after 3rd dose; heart blood showed moderate infection. After 2nd dose count was 12 per cent. |
| 12 | 57 | 4 × 0.008 | 0.2 | 35.0 | Animal died day after last dose; heart blood showed 35 per cent infection rate. Control monkey treated with another drug when showing 14 per cent infection; infection controlled and animal still alive 2 months later. |
| 13 | 63 | 4 × 0.01 | 13.0 | 16.3 | After 2 doses count was 8 per cent. Treatment with another drug failed to save animal. Untreated control died 2 days earlier. |
| 14 | 65 | 4 × 0.01 | 1.0 | 12.2 | Treated with another drug; animal alive. |

Experiment no. 7 was designed to facilitate the observation of the effects of the drug on the morphology of the parasites. The proportion of parasites showing such an effect was not greater than 5 per cent. In experiment no. 9, 0.008 gm. did not prevent the development of the parasites from 'rings' into normal mature schizonts which

days, after which the infection rate rose again to peak level. Animals not receiving further treatment with a more efficient drug died during the primary infection.

The conclusion is that mapharside has no significant action against *P. knowlesi*.

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# THE ACTION OF 2-CHLORO-7-METHOXY-5 (δ-DIETHYL-AMINO-BUTYL) AMINO-ACRIDINE ON SIMIAN MALARIA

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IN a previous paper it was observed by one of us (Basu and Bose, 1941) that although the toxicity (LD/50) of 2-chloro-7-methoxy-5 (δ-diethyl-amino-butyl) amino-acridine, in mice, was only slightly higher than that of 2-chloro-7-methoxy-5 (δ-diethyl-amino-iso-amyl) amino-acridine, the toxic effect of the former on cultures of *Paramecium caudatum* was found to be remarkably greater than that of the latter.

The toxicity of any compound on paramecia has been found to resemble closely its toxicity on other protozoal organisms (Kindler, 1938). The amyl-acridine derivative (under the trade name 'atebrin') is a well-known anti-malarial drug. Chopra and Das Gupta (1933) have demonstrated that this drug possesses a definite action against simian malaria. It was, therefore, considered to be of interest to investigate the action of the butyl-acridine, first, on *Plasmodium knowlesi* in the monkey, *Silenus rhesus*, and later, on the human plasmodia.

## *Methods*

The technique followed in the investigation was essentially that of Chopra and Das Gupta (1933), with the difference that the infection rate of the red blood cells was studied rather than the parasite rate. The drug was administered by intramuscular injection in sterile watery solution. Sets of doses varying from 25 mg. to 5 mg. were tried with the object of ascertaining the effect of smaller doses. The drug was administered in mild and heavy infections. The monkeys used were of roughly the same size, and those that were weighed scaled about 2 kilos.

## *Observations*

*Monkey no.* 1 (serial no. 12) showed 2.6 per cent infection of r.b.c.; given 20 mg. daily for 3 days—infection controlled. Relapse after 13 days; death 2 weeks later, 1.2 per cent infection of heart blood.

*Monkey no.* 2 (serial no. 24) showed 15.2 per cent infection of r.b.c.; given 25 mg. daily for 3 days—infection controlled. Death after 9 days; heart blood negative for parasites.

*Monkey no.* 3 (serial no. 31) showed 13.6 per cent infection of r.b.c.; given 25 mg. daily for 2 days—died after 3 days with 1.2 per cent infection of heart blood.

*Monkey no.* 4 (serial no. 25) showed 2.6 per cent infection of r.b.c.; given 10 mg. daily for 4 days—infection controlled. Relapse after 13 days, infection rising to 16.4 per cent; given 25 mg.—infection controlled and animal alive after 177 days with chronic infection.

*Monkey no.* 5 (serial no. 27) showed 2.4 per cent infection of r.b.c.; given 10 mg. daily for 5 days—infection controlled. Relapse after 17 days; treated with a different drug—animal died showing heavy infection with parasites.

*Monkey no.* 6 (serial no. 33) showed 15.0 per cent infection of r.b.c.; given 10 mg. daily for 5 days—infection controlled. No relapse observed after 116 days—animal alive.

*Monkey no.* 7 (serial no. 36) showed 13.2 per cent infection of r.b.c.; given 10 mg. daily for 5 days—infection controlled. Relapse after 14 days with chronic infection; no further treatment, animal alive after 110 days.

*Monkey no.* 8 (serial no. 37) showed 7.8 per cent infection of r.b.c.; given 5 mg. daily for 3 days—infection controlled. Relapse after 16 days with chronic infection; no further treatment, animal alive after 92 days.

The experiments show that the butyl-acridine derivative has a powerful parasiticidal action on *P. knowlesi*. There was evidence of degeneration in the parasites the day after the first injection. The parasite counts decreased rapidly, and after the third injection, the blood was generally found to be free of parasites. However, in four out of the five monkeys which have survived after treatment, a relapse occurred, though only two appeared to require further treatment. The relapse infection in monkey no. 5 was used for purposes separate from the present investigation. The smaller doses of 5 and 10 mg. appear to be as effective as the larger doses, and were tolerated better. The monkeys treated with the larger doses have died, while four out of five treated with the smaller doses are still alive after relatively long periods. The reader is reminded that *P. knowlesi* is highly pathogenic to rhesus monkeys.

## *Conclusion*

Preliminary experiments with the butyl-acridine derivative show that the drug possesses

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## *Summary and comments*

So far as we are aware, organic arsenicals have not been tried before in the treatment of simian malaria (*P. knowlesi*). Neither novarsenobillon ('N.A.B.') nor mapharside gave satisfactory results in the present trials, for in not one out of 14 monkeys treated with either of these drugs were parasites eradicated from the peripheral blood without the aid of other more efficient drugs. Both 'N.A.B.' and mapharside sometimes produced a temporary reduction in the infection rate of the red cells, and only in the cases of animals receiving 0.075 gm. or more of 'N.A.B.' did the infection rate show a continuous decline. Except with very large doses of 'N.A.B.' a parasiticidal action of the drugs was not much in evidence.

## REFERENCES

DAS GUPTA, B. M., and SIDDONS, L. B. (1943). *Indian Med. Gaz.*, **78**, 141.

GOLDMAN, D. (1938) .. *Amer. J. Med. Sci.*, **196**, 502.

YOUNG, M. D., and MCLENDON, S. B. (1939). *U. S. Pub. Health Rep.*, **54**, 1509.