CHEMOTHERAPEUTIC STUDIES ON PLASMODIUM INFECTION IN MONKEYS

No. V. ACTION OF TEBETREN

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IN previous papers (1933, 1934 and 1935) we have shown that monkeys of the species of *Silenus rhesus* are extremely susceptible to infection with *Plasmodium knowlesi*. The infection in these monkeys is so intense that it invariably proves fatal unless treated with effective anti-malarial drugs. As there are no chances of a spontaneous cure, chemotherapeutic studies on these animals have given a valuable means of testing the anti-malarial efficacy of these drugs. Further, the course of infection can be watched as it varies from day to day, and treatment can be started at any stage of the infection. Chopra and his co-workers have recently studied the effect of a number of synthetic anti-malarial remedies on these animals and have shown that within wide limits the results obtained are generally applicable to plasmodium infection in man.

Chopra and Das Gupta (1933) showed that the destructive action of atebrin on *P. knowlesi* was exceptionally powerful. Usually two doses of 0.025 gramme of the drug given intramuscularly or intravenously were sufficient to control a very heavy infection which may amount to a million parasites per c.mm. The drug affected equally the schizogony and the gametogony, and all phases of parasites rapidly disappeared from the peripheral blood under its action. In human malaria the drug was also found to be effective, its action being marked on the asexual forms, but less so on the sexual forms (Chopra *et al.*, 1933). In contra-distinction to the action of atebrin, quinine has a much less powerful immediate effect on these parasites. When the parasite count is low, *i.e.*, below 100,000 per c.mm., one dose (intramuscular or intravenous) may be effective in controlling the infection, but, when the count is high, 2 or 3 injections are necessary to control the infection (Chopra and Das Gupta, 1934). The advantage of quinine is that relapses do not as a rule occur after the treatment of the primary infection, and, even when they do occur, they are never fatal; whereas with atebrin fatal relapses in monkeys sometimes occur.

In this paper the result of studies with another synthetic drug, tebetren, is given. Tebetren was introduced by Messrs. Howard and Company, and chemically it is said to be methyl-hydrocupreine-methyl-acridine-dihydrocholate, in which quinine is combined with an acridine dye and united with a bile salt to render it less toxic. The drug is available in the form of tablets, each weighing 3 grains. Our object in these studies was to determine the effectiveness of this compound on *P. knowlesi* in monkeys and to study its toxicity, and also to make a comparative study of its effectiveness with other anti-malarial drugs such as atebrin, quinine, plasmochin, malarcan, etc.

Experimental

The animals used in our experiments were all *Silenus rhesus* varying in weight from 3 to 5 kilogrammes each. The animals were infected by subcutaneous injections of heavily parasitized blood from another monkey of the same species. As the pure drug was not available, each tablet of tebetren (3 grs.) was dissolved in distilled water 1 c.cm. of distilled water for each tablet), shaken and then centrifuged; the supernatant fluid containing the drug in solution was pipetted off and injected intramuscularly or intravenously. In most cases the treatment was commenced when the infection was heavy, the maximum being a million and a quarter parasites per c.mm. It was, however, found that heavy infections of over a million

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Oriel, G. H. (1931). *Proc. Roy. Soc. Med.*, Vol. XXIV, p. 1171.
Oriel, G. H. (1932). *Ibid.*, Vol. XXV, p. 1449.
Oriel, G. H., and Barber, H. W. (1930). *Lancet*, Vol. II, p. 231.
O'Neill, F. I., Moy, H. B., and Manwaring, W. H. (1925). *Journ. Immunol.*, Vol. X, p. 583.
Osman, A. A. (1929). *Lancet*, Vol. II, p. 1187.
Osman, A. A. (1929). *Brit. Med. Journ.*, Vol. I, p. 150.
Radhakrishna Rao, M. V. (1933). *Indian Journ. Med. Res.*, Vol. XXI, p. 141.
Rolleston, H. (1931). *Lancet*, Vol. II, p. 1227.
Schloss, O. M., and Anderson, A. (1922). *Proc. Soc. Exper. Biol. and Med.*, Vol. XX, p. 5.
Schloss, O. M., and Worthen, T. W. (1916). *Amer. Journ. Dis. Child.*, Vol. XI, p. 342.
Scott, W. J. M. (1928). *Journ. Exper. Med.*, Vol. XLVII, p. 185.
Spence, J. C., and Brett, P. C. (1921). *Lancet*, Vol. II, p. 1362.
Sussman, H., Davidson, A., and Walzer, M. (1928). *Arch. Intern. Med.*, Vol. XLII, p. 409.
Tallerman, K. H. (1923). *Quart. Journ. Med.*, Vol. XVII, p. 37.
Voegtlin, C., and Bernheim, B. M. (1911). *Journ. Pharm. and Exper. Therap.*, Vol. II, p. 507.
Wallace, G. B., and Diamond, J. S. (1925). *Arch. Intern. Med.*, Vol. XXXV, p. 698.
Walzer, A., and Walzer, M. (1928). *Arch. Dermat. and Syph.*, Vol. XVII, p. 659.
Walzer, M. (1927). *Journ. Immunol.*, Vol. XIV, p. 143.
Weil, R. (1917). *Ibid.*, Vol. II, p. 525.
Weil, R., and Eggleston, C. (1917). *Ibid.*, Vol. II, p. 571.
Wyman, L. C. (1928). *Amer. Journ. Physiol.*, Vol. LXXXVII, p. 29.

parasites per c.mm. always proved fatal in spite of prompt treatment. In the earlier experiments a course of 3 injections of tebetren in doses of 4½ to 6 grains daily was given by the intramuscular route, but in subsequent experiments injections were given for 4 days. In the intravenous series doses larger than 3 grains for a monkey of average weight (3 to 5 kilogrammes) proved fatal in a number of animals; doses of 3 grains were therefore given in the later experiments.

In the following tables the detailed observations of the effect of tebetren on these animals are recorded :

TABLES

No. I, *Silenus rhesus*, weight 4.540 kilogrammes, inoculated with 0.5 c.cm. blood from a heavily parasitized monkey (count not done) containing rings, trophozoites and schizonts. Incubation period 7 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
2-5-34	Rings	..	....
3-5-34	450,000	3 grains i.m.	Rings, growing trophozoites and schizonts. R.B.C. 6,200,000 per c.mm.
4-5-34	466,200	Do.	Trophozoites and schizonts. R.B.C. 5,180,000 per c.mm.
5-5-34	325,000	Do.	Parasites chiefly growing trophozoites. Infected cells showing Schüffner's dots. R.B.C. 5,000,000 per c.mm.
7-5-34	Scanty trophozoites.	Do.	Parasites showing evidence of degeneration. R.B.C. 4,320,000.
8-5-34	Scanty	Do.	Degenerating parasites.
9-5-34	Very scanty	Do.	Degenerating parasites. Marked basophilia. R.B.C. 3,800,000 per c.mm.
10-5-34	No parasites.	..	Marked basophilia. Normoblasts. R.B.C. 3,800,000 per c.mm.
14-6-34	Parasites (no count).	..	Rings, growing trophozoites and schizonts.
15-6-34	180,000	6 grains i.m.	Rings, growing trophozoites and schizonts. R.B.C. 3,000,000 per c.mm.
16-6-34	90,000	Do.	Normoblasts. R.B.C. 1,520,000 per c.mm.
17-6-34	15,000	Do.	Evidence of degeneration in the parasites.
18-6-34	No parasites.	Do.	Basophilia, normoblasts.

Tebetren was given by the intramuscular route in doses of 3 grains daily for 6 days. A low grade of infection persisted for about a month after the cessation of treatment of the primary infection. On 15th June, 1934, there was a relapse with a parasite count of 180,000 per c.mm.; a larger dosage, 6 grains daily for 3 days, completely controlled the infection. No parasites were found in the peripheral blood during an observation period of 5 months.

No. II, *Silenus rhesus*, weight 3.460 kilogrammes, inoculated with 1 c.cm. of heavily parasitized blood. Incubation period 8 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
25-5-34	140,000	6 grains i.m.	Rings and trophozoites. R.B.C. 4,600,000 per c.mm.
26-5-34	80,000	Do.	R.B.C. 4,000,000 per c.mm.
28-5-34	?	Do.	Evidence of degenerative changes in the parasites.
29-5-34 to 26-7-34	No parasites.	..	....

Two tablets of tebetren daily intramuscularly for 3 days completely controlled the infection. Degenerative changes of the parasites were marked after the second injection and they disappeared after the third injection. No parasites could be detected in the peripheral blood afterwards for an observation period of nearly 2 months. The monkey died on the night of 26th July, 1934, probably as a result of some intercurrent infection. Smear from heart's blood showed no parasites.

No. III, *Silenus rhesus*, weight 3.510 kilogrammes, inoculated with 0.5 c.cm. blood containing 275,000,000 parasites. Incubation period 4 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
11-6-34	100,000	..	Rings and growing trophozoites.
12-6-34	1,000,000	6 grains i.m.	Rings, trophozoites and schizonts.
13-6-34	425,700	Do.	Do.
14-6-34	23,350	Do.	Degenerating parasites. R.B.C. 4,670,000 per c.mm.

Treatment was started in this case when the parasite count was very high, *i.e.*, 1,000,000 parasites per c.mm. The parasites were nearly halved 24 hours after the first injection and completely disappeared from the peripheral blood after a course of 3 injections (6 grains daily for 3 days). A relapse occurred 12 days after this like that observed in cases treated with atebrin, and proved fatal. A smear from heart's blood showed that almost every red cell was infected, many showing multiple infection. This is the only animal in our series of experiments in which a relapse proved fatal.

No. IV, *Silenus rhesus*, weight 3.695 kilogrammes, inoculated with 1 c.cm. blood containing 405,900,000 parasites. Incubation period 5 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
26-6-34	778,000	6 grains i.m.	Parasites mostly rings. R.B.C 5,190,000 per c.mm.
27-6-34	625,500	Do.	All forms of parasites, rings, trophozoites, schizonts and gametocytes. R.B.C. 4,270,000 per c.mm.
28-6-34	Scanty	Do.	Evidence of degenerative changes in the parasites. R.B.C. 4,190,000 per c.mm.
29-6-34	No parasites.	..	Commencing basophilia. Demilune cells.
9-7-34	Scanty	..	Rings.
12-7-34	357,000	6 grains i.m.	Mostly rings. Few growing trophozoites. R.B.C. 5,100,000 per c.mm.
13-7-34	414,000	Do.	Mostly growing trophozoites, few rings. Evidence of degeneration in the parasites. Commencing basophilia. R.B.C. 4,600,000.
14-7-34	67,500	Do.	Degenerative changes in the parasites.
16-7-34	No parasites.	Do.	Basophilia, normoblasts.

This animal received a course of 3 injections (6 grains daily) when the parasite count was fairly high. A relapse occurred in ten days, all stages of parasites being found in the peripheral blood. A longer course of 4 injections was given (6 grains daily); the parasites disappeared and were not found again in the peripheral blood during an observation period of 4 months.

No. V, *Silenus rhesus*, weight 3.720 kilogrammes, inoculated with 0.5 c.cm. blood containing 173,100,000 parasites. Incubation period 5 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
3-7-34	130,000	..	Parasites mostly rings.
4-7-34	700,000	6 grains i.m.	Rings and growing trophozoites. R.B.C. 5,000,000 per c.mm.
5-7-34	1,009,800	Do.	Mostly growing trophozoites and schizonts. R.B.C. 4,590,000 per c.mm.
6-7-34	80,000	Do.	Parasites showing degenerative changes.
7-7-34	No parasites.	Do.	Commencing basophilia, normoblasts.

Treatment was started when the parasite count was fairly high, *i.e.*, 700,000 parasites per c.mm. A course of 4 injections (6 grains daily) was given from the beginning instead of three as in the previous experiments. In this case the first injection had no effect on the parasites and the count rose above 1 million on the second day; it came down subsequently and the parasites disappeared after 3 injections. Degenerative changes in the parasites were well marked after 2 doses. This animal was under observation for more than 4 months after the cessation of treatment, and frequent examinations of the blood did not show any parasites. In this respect the action of tebetren resembled that of quinine.

No. VI, *Silenus rhesus*, weight 5 kilogrammes, inoculated with 1 c.cm. blood containing 700,000,000 parasites. Incubation period 5 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
11-7-34	931,000	6 grains i.m.	Rings and growing trophozoites. R.B.C. 4,900,000 per c.mm.
12-7-34	491,000	Do.	All forms of parasites including gametocytes. R.B.C. 4,910,000 per c.mm.
13-7-34	Scanty	Do	Evidence of degenerative changes in the parasites.
14-7-34	Do.	Do.	Do.
15-7-34 to 15-11-34	No parasites.	..	....

This monkey was given a course of 4 injections, 6 grains daily. After 2 injections the parasites showed degenerative changes, their number being considerably reduced; scanty degenerating forms were found for the next two days. Subsequently the peripheral blood remained free of parasites (similar to monkeys II and V).

No. VII, *Silenus rhesus*, weight 4.560 kilogrammes, inoculated with 0.5 c.cm. blood containing 221,200,000 parasites. Incubation period 5 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
20-8-34	260,000	..	Rings and growing trophozoites.
21-8-34	688,500	6 grains i.m.	All forms of parasites. R.B.C. 5,100,000 per c.mm.
22-8-34	1,260,000	Do.	All forms of parasites, apparently healthy. R.B.C. 4,500,000 per c.mm.

This monkey was given the drug intramuscularly when the parasite count was fairly high. The count rose to above 1 million in spite of treatment (1,260,000 parasites per c.mm.), a condition hardly compatible with life.

No. VIII, *Silenus rhesus*, weight 3 kilogrammes, inoculated with 0.5 c.cm. blood containing 344,250,000 parasites. Incubation period 5 days.

In this monkey treatment was started on 27th August, 1934, when the parasite count was above 1 million (1,200,000 parasites per c.mm.) in order to see whether the drug had any effect in such a heavy infection; 4½ grains of tebetren was given intramuscularly. The monkey died on the same night. Smears from liver and heart's blood showed that these organs were crammed with parasites, nearly 80 per cent of the red cells being infected.

No. IX, *Silenus rhesus*, weighing 3.100 kilogrammes, was inoculated with 0.25 c.cm. blood containing 300,000,000 parasites. Incubation period 5 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
3-9-34	561,000	3 grains i.m.	Rings and growing trophozoites. R.B.C. 5,100,000 per c.mm.
4-9-34	133,500	Do.	All stages of parasites. R.B.C. 4,450,000 per c.mm.
5-9-34	Scanty	Do.	Evidence of degenerative changes in the parasites.
6-9-34	Do.	Do.	Evidence of degenerative changes in the parasites. Basophilia, normoblasts.

This animal received a course of 4 injections (3 grains daily). Very scanty parasites however persisted in the blood for a week after cessation of treatment; on the eighth day the count again rose to 120,000 parasites per c.mm. The multiplication of parasites was however less rapid and the count gradually came down without further treatment. Subsequent to this the peripheral blood remained mostly free of parasites.

No. X, *Silenus rhesus*, weight 4.730 kilogrammes, inoculated with 0.5 c.cm. blood containing 70,000,000 parasites. Incubation period 7 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
2-6-34	300,000	4½ grains i.v.	Rings and growing trophozoites. R.B.C. 5,010,000 per c.mm.
3-6-34	160,000	Do.	R.B.C. 4,100,000 per c.mm.
4-6-34	Scanty	Do.	Degenerating parasites.
5-6-34	Do.	..	....
6-6-34	Do.	..	....
8-6-34	Parasites ++ (count not done).	..	....
9-6-34	219,000	4½ grains i.v.	....
11-6-34	22,500	Do.	Few rings, mostly trophozoites.
12-6-34	Scanty	Do.	Degenerating parasites.

In this animal the infection was controlled with doses of 4½ grains daily for 3 days intravenously. Though the primary infection was controlled with this course of treatment, it was not sufficient to prevent the rapid multiplication of parasites within a very short time (4 days). The parasites again appeared in the peripheral blood 12 days after the second course of tebetren, but this time they multiplied less rapidly and gradually disappeared even without treatment. This animal was under observation for a period of over 4 months after the cessation of treatment, and the peripheral blood remained free of parasites during the period.

No. XI, *Silenus rhesus*, weight 3 kilogrammes, inoculated with 0.5 c.cm. blood containing 150,000,000 parasites. Incubation period 3 days.

This monkey died within five minutes of an intravenous injection of 4½ grains of tebetren. As this dose was toxic, the dosage by the intravenous route was reduced in the subsequent experiments.

No. XII, *Silenus rhesus*, weight 3.880 kilogrammes, inoculated with 1 c.cm. blood containing 1,000,000,000 parasites. Incubation period 7 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
20-6-34	405,900	3 grains i.v.	Rings and growing trophozoites. R.B.C. 4,510,000 per c.mm.
21-6-34	500,800	4½ grains i.v.	Rings and growing trophozoites. R.B.C. 4,590,000 per c.mm.
22-6-34	480,000	Do.	All forms of parasites including gametocytes. Evidence of degenerative changes. R.B.C. 4,000,000 per c.mm.
23-6-34	40,000	Do.	Degenerating parasites.
24-6-34	Scanty parasites.	..	....
25-6-34	No parasites.	..	....
1-7-34	155,200	4½ grains i.v.	Rings and trophozoites.
2-7-34	83,750	Do.	Parasites appear to be healthy.
3-7-34	Scanty	4½ grains i.m.	The injection was given intramuscularly because of the difficulty of finding a suitable vein after repeated i.v. injections.
4-7-34	No parasites.	6 grains i.m.	Basophilia, poikilocytosis, normoblasts, etc.
19-7-34	Rings ++	..	....
20-7-34	118,800	6 grains i.m.	Mostly growing trophozoites, a few schizonts. R.B.C. 4,000,000 per c.mm.
21-7-34	68,200	Do.	Evidence of degeneration. R.B.C. 3,410,000 per c.mm.
22-7-34	No parasites.	Do.	Basophilia, normoblasts.
23-7-34	Do.	Do.	Do.

This animal was given a course of 4 injections intravenously. The parasites disappeared after the

fourth injection (usually after 2 injections by the intramuscular route). A relapse or recrudescence occurred 5 days after the treatment of the primary infection and again a fortnight after the second course of tebetren, for which tebetren was given partly intravenously and partly by the intramuscular route. A low grade of infection persisted for about 2 months but the parasites never multiplied rapidly. Subsequent to this the peripheral blood showed no parasites.

No. XIII, *Silenus rhesus*, weight 3.610 kilogrammes, inoculated with 0.5 c.cm. blood containing 389,000,000 parasites. Incubation period 6 days.

This monkey received 4½ grains of tebetren intravenously. Immediately after the injection the animal had convulsions with spasmodic respiration and died within half an hour (*see* also monkeys XI and XIV).

No. XIV, *Silenus rhesus*, weight 4.290 kilogrammes, inoculated with 0.5 c.cm. blood containing 465,500,000 parasites. Incubation period 7 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
19-7-34	395,200	4½ grains i.v.	Mostly rings, few growing trophozoites.
20-7-34	125,000	Do.	R.B.C. 6,080,000 per c.mm. Rings and trophozoites. The animal had convulsions of the whole body immediately after the injection. Died after ten minutes.

It has already been shown that a dosage larger than 3 grains intravenously proves toxic for these animals. In this case though the parasite count came down appreciably after the first injection, the animal succumbed after the second dose.

No. XV, *Silenus rhesus*, weight 4.100 kilogrammes, inoculated with 0.5 c.cm. blood containing 197,600,000 parasites. Incubation period 7 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
27-7-34	..	..	Rings and growing trophozoites.
28-7-34	276,000	3 grains i.v.	Rings and trophozoites. R.B.C. 6,140,000 per c.mm.
29-7-34	770,900	Do.	All forms of parasites including gametocytes, apparently healthy. R.B.C. 5,930,000 per c.mm.
30-7-34	58,500	Do.	Evidence of degenerative changes in the parasites. R.B.C. 4,000,000 per c.mm.
31-7-34	Scanty	Do.	Degenerating parasites. R.B.C. 3,800,000 per c.mm.
3-8-34	Do.	Do.	....

In this monkey the dose of tebetren by the intravenous route was reduced to 3 grains and was well tolerated. The parasite count came down appreciably after 3 injections, but a low grade of infection persisted for more than 6 months for which no treatment was necessary as the parasites never multiplied rapidly.

No. XVI, *Silenus rhesus*, weight 4.159 kilogrammes, inoculated with 0.5 c.cm. blood containing 138,000,000 parasites. Incubation period 6 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
4-8-34	102,000	3 grains i.v.	Mostly rings and growing trophozoites. R.B.C. 5,100,000 per c.mm.
5-8-34	162,000	Do.	All stages of parasites. R.B.C. 5,400,000 per c.mm.
6-8-34	42,000	Do.	Degenerating parasites. R.B.C. 4,200,000 per c.mm.
7-8-34	20,000	Do.	Degenerating parasites.
8-8-34	Scanty	..	Do.

In this animal the infection was controlled after the fourth injection of tebetren intravenously. Rapid multiplication of the parasites occurred from the fifth day after cessation of treatment of the primary infection which was controlled with 3 more intravenous doses (3 grains daily). Though a relapse occurred 18 days after this, the multiplication of the parasites was less rapid and they disappeared without any further treatment.

No. XVII, *Silenus rhesus*, weight 5.216 kilogrammes, inoculated with 0.5 c.cm. blood containing 51,000,000 parasites. Incubation period 7 days.

Date	Parasite count per c.mm.	Treatment	REMARKS
13-8-34	Rings and trophozoites ++	..	....
14-8-34	442,400	3 grains i.v.	All stages of the parasites. R.B.C. 5,530,000 per c.mm.
15-8-34	90,000	Do.	All stages of the parasites. R.B.C. 4,500,000 per c.mm.
16-8-34	174,000	Do.	Parasites are apparently healthy. R.B.C. 4,350,000 per c.mm.
17-8-34	Scanty	Do.	Degenerating parasites.
18-8-34 to 21-8-34	Do.	..	....
23-8-34	280,000	..	Mostly rings. Normoblasts, basophilia. R.B.C. 3,500,000 per c.mm.

No. XVII, *Silenus rhesus*—concld.

Date	Parasite count per c.mm.	Treatment	REMARKS
24-8-34	96,000	..	Mostly rings and trophozoites. Basophilia, normoblasts and pigmented leucocytes. Some of the parasites are degenerating.
25-8-34	180,000	..	Do.
26-8-34	55,000	..	Do. R.B.C. 2,500,000 per c.mm.
27-8-34	150,000	..	....
28-8-34	75,000	..	....
29-8-34	37,500	..	....
30-8-34	10,000	..	....
1-9-34	Scanty	..	Degenerating parasites. Basophilia, normoblasts.

In this monkey the parasite count came down after 3 intravenous injections, scanty parasites however persisting. Five days after the cessation of treatment, the parasites multiplied rapidly and the count rose to 280,000 parasites per c.mm. No treatment was given for this; though the infection persisted for more than a month, the parasite count gradually came down. Later during an observation period of about 3 months, the peripheral blood remained mostly free of parasites (*see* also monkeys IX, X and XVI).

Discussion

A perusal of the foregoing tables will show that in monkeys I to IX tebetren was administered intramuscularly while in monkeys X to XVII it was given intravenously. The drug undoubtedly has a destructive effect on *Plasmodium knowlesi* infection in these animals whether it is given by the intramuscular or the intravenous route. Dosage of 4½ to 6 grains daily for 2 to 3 days by the intramuscular route and 3 grains daily intravenously for 3 to 4 days controlled a heavy infection which amounted to as much as a million parasites per c.mm. In the majority of the animals its action was immediate; the parasites usually decreased in numbers after the first injection or, even when they did not decrease, their multiplication stopped or was considerably inhibited. In this respect the drug differed somewhat from quinine which, in our experiments described in a previous paper (1934), did not so promptly stop or inhibit the growth of the parasites after the first injection, but often appeared actually to accelerate their multiplication. This increase in the number of parasites in the peripheral blood was also observed after intravenous injection of quinine in man by Chopra and his coworkers (1932). It will also be observed that in some animals of this series, after a full course of treatment of the primary infection, the plasmodia disappeared from the peripheral blood entirely (nos. II, V and VI), while in others they reappeared after an interval, producing a relapse (nos. I, III, IV, XII and XVI) and in no. III the relapse proved fatal in spite of treatment.

Dosage.—Monkeys weighing between 3 to 5 kilogrammes tolerated a dose of 4½ to 6 grains dissolved in 1½ to 2 c.cm. of distilled water by the intramuscular route without any untoward effects; no local reactions were observed. The number of injections necessary to free the peripheral blood from plasmodia varied from 3 to 4, and this was effective in controlling a heavy infection (nos. III, IV, V and VI). By the intravenous route a dose of 3 grains could be administered safely, but doses larger than this proved fatal and three animals died after injections of 4½ grains of tebetren (nos. XI, XIII and XIV). Doses of 3 grains in animals weighing 4 to 5 kilos. appear to be safe and, when compared with the effective therapeutic dose of 24 to 36 grains (8 to 12 tablets a day) for an adult human being weighing 60 to 80 kilos., show that the toxicity of the drug is fairly low.

Intramuscular and intravenous injections.—A study of tables I to IX shows that after a single intramuscular injection of tebetren in most of the cases the plasmodia showed degenerative changes, were quickly destroyed and their number in the peripheral blood showed a considerable decrease. The reduction in the number of parasites appeared after the first injection but was definite and considerable after the second injection. In this respect the effect of tebetren would appear to approach that of atebrin, which in our experiments reported in a previous paper (1933) produced a remarkable decrease in the number of parasites as well as marked degenerative changes in their cytoplasm almost immediately. Though the action of this drug was not so rapid as that of atebrin, the results were fairly constant and a very heavy infection appeared also to be more amenable to treatment with tebetren than quinine. Intramuscular injections of quinine, it will be remembered, have a gradual action on the parasites and, as a rule, there is no remarkable decrease in their numbers for 24 hours after the first injection or even longer.

The state of affairs after intravenous injection of tebetren, however, was different. Whereas atebrin produced similar effects on the parasites whether given by the intramuscular or the intravenous route, the effect of tebetren on the plasmodia when given intravenously was much less evident. The effects observed were variable and undoubtedly much weaker after the first injection; sometimes even after the second injection hardly any changes were noticed. As a matter of fact after the first injection in most of the animals (nos. XII, XV and XVI) there was an actual increase in the number of parasites in the peripheral blood, and as a rule 3 injections were necessary to

reduce appreciably the number of plasmodia. The parasite count increased on the second day in monkeys XV and XVI, decreased in monkey XVII but increased again on the third day; the count in no. XII remained more or less steady for 3 days while only in no. X it came down appreciably from the second day onward. This effect of tebetren may be due to rapid excretion of the drug when given intravenously so that the contact of the drug with the parasites is of shorter duration.

Comparative action of atebrin, quinine and tebetren

It has already been stated that atebrin has a powerful immediate effect on *Plasmodium knowlesi*. Even when the parasite count is high, one dose of this compound produces an enormous reduction in the number of the plasmodia and the infection is controlled by whatever route the drug is administered, intramuscular or intravenous. In the case of quinine one dose is only effective when the parasite count is low but in heavy infections two or more injections are necessary. Tebetren would appear to stand between quinine and atebrin in its action on this plasmodium in so far as the reduction in the number of parasites is concerned. Generally the parasite count is nearly halved 24 hours after an intramuscular injection of atebrin and degenerative changes are evident after the second injection. By the intravenous route the effects in case of tebetren are less conspicuous and two or three injections are necessary to bring down the parasite count, and in this respect it bears a closer resemblance to quinine.

So far as relapses are concerned, tebetren appears to possess an advantage over atebrin. After five days' intensive treatment with atebrin in fairly large doses, the plasmodia invariably reappeared in 10 to 15 days and multiplied with the same rapidity as in the primary attack causing death of the animal if prompt treatment was not given, whereas with tebetren though a relapse or recrudescence occurred in some animals in 5 to 12 days, it did not prove fatal except in no. III. In some of the relapses the animals were kept without treatment intentionally, and the parasites often disappeared spontaneously (nos. IX, X, XVI and XVII). In nos. II, V and VI the parasites disappeared altogether from the peripheral blood after an intensive treatment of the primary infection with this compound and in monkey XV the parasites were scanty, never multiplied and the animal remained healthy without further treatment.

Tebetren chemically is said to be a methyl-hydrocupreine-methyl-acridine - dihydrocholate, *i.e.*, a compound in which quinine is combined with acridine from which atebrin is derived. The destructive action of tebetren on *P. knowlesi* infection appears to combine the virtues of quinine and atebrin. So far as the control of infection is concerned, it has a powerful and rapid destructive action on the plasmodia, thus resembling atebrin; reduction in the number of the parasites in the peripheral blood is more rapid than with quinine. With regard to relapses the effects on these animals were very much like those produced in quinine-treated animals inasmuch as the parasites either disappeared spontaneously after a relapse or if they multiplied the multiplication was not so intensive as to cause death of the animal except in one instance. The action of tebetren in human malaria is under investigation but while expressing no opinion regarding the efficacy or otherwise of tebetren on malarial infection in man, we are justified in concluding that this drug has a fairly powerful action on *P. knowlesi*. Intramuscular injections are more effective and better tolerated than intravenous injections, this being probably due to more prolonged contact of the drug with the parasites.

Summary

The results of investigations regarding the action of tebetren on plasmodium infection in *Silenus rhesus* are given. Tebetren is a synthetic drug in which quinine is said to be combined with an acridine dye and bile salts are added to render it less toxic. The results of the experiments on this monkey indicate that tebetren is a fairly efficacious drug in the treatment of infections with *Plasmodium knowlesi*. A dose of 4½ to 6 grains daily for 2 days by the intramuscular route and 3 grains daily intravenously for 3 days controlled fairly heavy infections amounting to as much as a million parasites per c.mm. In so far as the decrease in the number of parasites in the peripheral blood is concerned, tebetren appears to be intermediate in action between atebrin and quinine. The parasite count is nearly halved 24 hours after an intramuscular injection and degenerative changes in the parasites are evident after the second injection. By the intravenous route, however, the effects are less marked and two or three injections are necessary to bring down the number of parasites in the blood appreciably; in this respect its action resembles quinine.

So far as relapses are concerned, the effects observed resembled more or less those produced in quinine-treated monkeys; the parasites either disappeared spontaneously, or, if they multiplied, the multiplication was slow and except in one case death did not occur. The drug, it seems, combines the virtues of atebrin and quinine and gives promise of being a useful remedy in the cure of malarial infection. It is on trial on human cases of malaria in the Carmichael Hospital for Tropical Diseases.

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THE PASSAGE OF HOOKWORMS AFTER TREATMENT

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IN industrial practice the impossibility of keeping under control large numbers of coolies for a prolonged period after mass treatment for hookworm infection has led to the employment of various rough and ready methods of assessing the efficacy of such treatment. One of the commonest of these is to examine for the presence of hookworms the first stool passed after treatment. The senior author has several times been asked what he considered to be the value of this method, and while expressing the opinion it was probably not of much use he always had the feeling he might be wrong, because as far as he knew no definite data on the point were available. It was accordingly decided to investigate this question.

The following method was adopted in the inquiry. Only hospital in-patients were made use of because sufficient supervision of out-patients was impracticable. All the patients were treated by our usual routine method; they were given 3 c.cm. of tetrachlorethylene and 1 c.cm. of oil of chenopodium shaken up in two ounces of saturated solution of magnesium sulphate. The treatment was given at 6 a.m. and from this hour until 6 p.m. on the same day all the stools passed were kept separate and individually examined for hookworms by washing through a fine sieve, the times at which all the stools in this first twelve hours were passed was also noted. From 6 p.m. until 6 a.m. the following morning any stools passed were examined for each patient as a whole and the same was done for the second, third and fourth twenty-four-hour periods respectively.

One hundred cases treated for hookworm infection in the Carmichael Hospital for Tropical Diseases were dealt with in the above manner. Many of these were only light infections as they had been admitted for conditions other than hookworm infection and the latter was only found in the course of the usual routine examinations. It was noticed that the proportion of lightly infected persons failing to pass worms in the first stool was slightly higher than that of those more heavily infected; this point is discussed below.

Seventy-three patients were cured, but as cure or failure to cure had no bearing at all on the passage of worms in the first stool this aspect has been ignored and cured and uncured cases are discussed together. Cure was considered established if the patient showed no eggs in a direct centrifugal flotation preparation ten days after treatment. Egg counts were done on all the patients before treatment with the idea of gaining an approximate estimate of the number of worms that might be anticipated from each case, but as they showed absolutely no correlation with the number of worms subsequently passed they have not been considered.

Altogether 42 of the patients passed no worms in the first stool and 22 passed the first stool between 6 and 6-30 a.m., that is within half an hour of the treatment being given, and eight of these 22 passed worms in the stool. Of the 14 who did not pass worms in the first stool 5 passed their stools between 6 and 6-15 so it is considered better not to include these 5 as failures to pass worms, as it was such a short time after treatment, therefore of this group we can say that 8 passed worms and 9 failed to do so. As a check on the time that worms might be expected to appear in the stools after treatment those passing their first stool between 6-30 and 8 a.m. were taken separately; there were 26 in this group and 13 passed worms while 13 did not do so. A further check was the taking of those who passed their first stools between 8 and 10 a.m., there were 25 in this group and only 10 of them passed worms in the first stool. From these observations it seems safe to assume that the chance of finding worms in stools passed half an hour after treatment is as good as when the stool is retained for over two hours. Therefore, excluding the 5 who passed stools earlier than 6-20 a.m., we can say that 39 persons failed to pass worms in the first stool, and 27 of these passed less than a total of 20 worms.

It was mentioned above that the proportion of failures to pass worms in the first stool was somewhat greater among the light infections, so a division was made between those passing 1 to 19 worms and those passing 20 or more. Out of 55 lightly infected 28 passed worms and 27 did not (this includes 3 who passed stools

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REFERENCES

Chopra, R. N., and Das Gupta, B. M. (1933). Studies on the Action of Atebrin in Plasmodium Infection of Monkeys. *Indian Med. Gaz.*, Vol. LXVIII, p. 493.

Chopra, R. N., and Das Gupta, B. M. (1934). Studies on the Action of Quinine in Monkey Malaria. *Ibid.*, Vol. LXIX, p. 195.

Chopra, R. N., Das Gupta, B. M., and Sen, B. (1932). A Fatal Case of Severe Malignant Tertian Malaria. *Ibid.*, Vol. LXVII, p. 680.

Chopra, R. N., Das Gupta, B. M., and Sen, B. (1933). Atebrin in the Treatment of Indian Strains of Malaria. *Ibid.*, Vol. LXVIII, p. 425.

Chopra, R. N., Ganguli, S. K., and Roy, A. C. (1935). On the Relationship between the Quinine Concentration in the Circulating Blood and Parasite Count in Monkey Malaria. *Ibid.*, Vol. LXX, p. 62.

Chopra, R. N., Mukherjee, B., and Campbell, H. G. M. (1933). The Pharmocological Action and Anti-Malarial Properties of Anhydrocotarnine-resorcinol-hydrochloride. (A derivative of narcotine.) *Indian Journ. Med. Res.*, Vol. XXI, p. 255.