EXPERIMENTAL STUDIES ON APE MALARIA WITH REFERENCE TO ITS USE IN MALARIA THERAPY FOR NERVOUS CONDITIONS.

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Since the successful transmission of monkey malaria to human beings by Knowles and Das Gupta (1932) it has been tried by some workers in the treatment of nerve syphilis.

Van Rooyen and Pile (1935) treated 12 cases of general paralysis of the insane. These observers noted that there existed a marked variability with regard to the degree of susceptibility to infection with this parasite. Although the majority of the individuals inoculated with the blood of infected monkeys were readily infected, a few showed marked refractoriness in that the infection failed to establish itself in these individuals. This resistance was particularly noticeable in the case of patients who had previously received a course of benign tertian malaria therapy. These workers were unable to assess the value of this mode of therapy, for their observations on these cases were very short. But they hoped that monkey malaria might ultimately prove to be an effective substitute for *P. vivax* in the treatment of G.P.I. According to these observers atebrin has little or no effect on the reduction of temperature or destruction of the parasites (*P. knowlesi*).

To determine whether monkey malaria could be employed for malaria therapy in nervous cases we have made the following inoculation experiments.

Case I.—This is a case of optic atrophy with a history of syphilis and moderately positive Wassermann reaction.

History of inoculations: On 3-1-36 he was inoculated with 0·5 c.c. of blood showing a massive infection with *P. knowlesi* (8,240 parasites per 100 leucocytes). Temperature began to rise on the 15th day of inoculation and persisted for a week. Fever was of the quotidian type and varied from 102–104°F. Infection died out spontaneously. He was now examined by the ophthalmologist who reported that the condition of the eye was the same as before, but the patient felt that his eye-sight had appreciably improved. About 9 months later he came under our observation again. It was now decided to give him another course of fever. Accordingly he was re-inoculated with *P. knowlesi*. A much bigger dose than in the previous inoculation was used. As the patient showed absolute resistance to *P. knowlesi*, he was injected with *P. vivax*. Infection was readily established. After he had 9

rigors the patient was given a course of quinine treatment. His eyes were examined again, but no improvement was observed. About 6 weeks after the experimental infection with *P. vivax*, he came back with fever showing typical benign tertian periodicity and scanty parasites (*P. vivax*) in the blood. It is not possible to say whether this was a relapse or whether he contracted a fresh infection during his stay at Jessore, a place which is reputed to be a hot-bed of malaria in Bengal.

Case II.—The patient was suffering from advanced optic atrophy with derangement of mental condition—restlessness alternating with depression and disorder of speech. Wassermann reaction was strongly positive.

History of inoculations: On 30-1-36 he was inoculated with 0·8 c.c. of blood from a *S. rhesus* heavily infected with *P. knowlesi* (4,200 parasites per 100 leucocytes). On the 14th day of inoculation temperature started rising and blood showed scanty parasites in the thick films. Three days later temperature rose to 104·6°F., although the parasite count was low (529 growing trophozoites and schizonts per 1,000 leucocytes). At this stage the patient became semi-conscious with marked rigidity of the neck. So it was decided to terminate the fever. A dose of atebrin (0·1 gram) was given by the mouth. As the patient showed sensitiveness to atebrin as evidenced by nausea and yellow colouration of the skin 0·5 gramme of quinine was given by intramuscular injection. Within 24 hours temperature came down to normal and all the symptoms passed off. On 4-4-36 he was re-inoculated with a large dose of *P. knowlesi* without infection resulting. On 24-9-36 he was inoculated with *P. vivax* and was readily infected. There was irregular pyrexia for 11 days, which subsided spontaneously. Again on 11-2-37 he was inoculated with the blood containing a mixed infection with *P. vivax* and *P. falciparum*. After a short incubation period of 8 days he developed high temperature (105·2°F.), showing only the rings of *P. falciparum*. Fever was controlled with 0·2 grammes of atebrin daily for 3 days. On 12-3-37 fever recurred, associated with only *P. vivax* (almost all phases) in the blood. After 6 rigors with high temperature (rising up to 104°F. or even more) further progress of the infection was checked by a full course of atebrin. There was no noticeable improvement in the patient's condition.

Case III.—The case is one of primary optic atrophy with negative Wassermann reaction.

History of inoculations: On 6-4-36 he was inoculated with 2 c.c. of blood from a *S. rhesus* heavily parasitized with *P. knowlesi*. He started getting fever on 12-4-36. Parasites first appeared on 18-4-36. Temperature never rose above 102°F. and came down to normal on 26-4-36. No antimalarial treatment was given. On 1-6-36 he was inoculated with 4 c.c. of blood (1,280 parasites per 1,000 leucocytes) from a case of benign tertian malaria. On 18-6-36 scanty parasites were found in the thick smears. There was a slight rise of temperature on 10-6-36. Since this date he became completely afebrile. In March 1937, he was inoculated for the second time with 5 c.c. of blood from

a very heavily infected *S. rhesus* (80 per cent of the animal's red cells infected with one or more parasites). Even such a massive dose failed to produce an infection.

Case IV.—The patient was suffering from mental deterioration—delusion of being constantly persecuted by his relatives. Wassermann reaction was strongly positive.

History of inoculations: On 1-5-36 he was inoculated with a massive dose of *P. knowlesi* from a heavily infected *rhesus* monkey. He started getting fever on 11-5-36 and the parasites were first detected in the blood two days later. After the fever had continued for 10 days it was considered necessary to cut short the attack as the patient was much pulled down and complained of cardiac discomfort. Accordingly he was given a dose of 0·2 gramme of atebrin by the mouth and the temperature came down to normal within the next 12 hours. There was distinct amelioration of his mental condition, but it did not last long. Two months later, he was again brought to one of us (B.M.D.) who thought that another course of febrile attack might be tried with benefit. So he was inoculated with 5 c.c. of blood from a case of benign tertian malaria showing moderate infection. Fever started on the 13th day of inoculation. After the patient was allowed to have 8 well-marked rigors the infection was cut short by a single dose of 5 grains of quinine. Apparently not much benefit was derived by the patient from this course of fever. About 3 months later the patient came under our observation again and his relations insisted on giving him another course of malaria therapy. On 4-5-37 we injected him with the blood from a patient who was suffering from benign tertian malaria, blood smears showing chiefly growing trophozoites. On the 9th day of inoculation he had high temperature and curiously enough the blood films showed only rings of *P. falciparum*. (The specific identification of the parasite was confirmed by cultural method). Next day his temperature rose to 104·8°F. and he became drowsy. At this stage we checked the further progress of the infection by a course of atebrin.

Case V.—A healthy volunteer with no history of fever within the past 10 years.

History of inoculations: On 5-5-37 he was inoculated with 4·5 c.c. of blood from a monkey showing a massive infection with *P. knowlesi*. After an incubation period of 9 days he started getting fever. Parasites could not, however, be detected on this date. Fever continued and varied from 101°F. to 104°F. As the infection showed no tendency to die out spontaneously it was arrested by a short course of atebrin. After only two doses (0·1 gramme each) the temperature came down to normal. The parasite count was never high, maximum count being 320 parasites per 1,000 leucocytes. On 1-2-37 he was inoculated with a large dose of *P. knowlesi*, but there was no response.

Case VI.—This case suffered from an attack of malignant tertian malaria and was treated with a full course of atebrin and plasmoquine.

History of inoculation: Five weeks after he had his last dose of combined atebrin and plasmoquine he was given a large dose of *P. knowlesi*. From the 7th day of inoculation his blood films were examined daily for two weeks. As no evidence of infection was noticed he was again inoculated with the same parasite and was kept under observation for a month. During this period he neither showed parasites in the blood nor had he any rise of temperature.

Discussion.

Sinton and Mulligan (1933) have made a series of observations with a number of strains of *P. knowlesi* in connection with their studies in immunity in malaria, and noted that superinfection with a homologous strain of *P. knowlesi* in *S. rhesus* failed to produce a re-infection, and if re-infection was produced its effects were so mild that it could only be recognised by very slight transient increase in the number of parasites in the animal's blood. They have also shown that an infection with one strain does not appear to protect against superinfection with a different strain of the parasite. In reviewing the literature on the subject of superinfection in human malaria, these authors observe that the evidence at present available suggests that the duration of tolerance to re-infection is largely dependent on the continued presence of infecting organisms in the body. For what length of time and in what degree of efficiency this tolerance persists in the human subject after the infection has been cured has not been definitely proved. There is, however, evidence suggesting that in the absence of re-infection after cure the tolerance rapidly diminishes. The experiments recorded above show that an infection with *P. knowlesi* probably confers complete immunity to further inoculation with the parasite (cases I–III). The immunity produced in man following an infection with *P. knowlesi* may be regarded as true immunity and not an 'infection immunity' which is associated with the presence of parasites in the body of the host as is supposed to be the case in human malaria.

Van Rooyen and Pile (1935) pointed out that the individuals who previously received a course of *P. vivax* malaria therapy were resistant to infection with *P. knowlesi*. This observation has also been confirmed by us (case VI). On the other hand a previous infection with *P. knowlesi* does not protect against an infection with human malaria (cases I–V). Three neuro-syphilitics listed above were infected with *P. knowlesi*. Besides, they received one or more attacks of human malaria. Apparently none seemed to derive any appreciable benefit from either form of malaria therapy. It is probable that the malaria suffered by these patients was not sufficient, although one patient ran a course of continued fever for 10 days (*P. knowlesi* infection). Two months later he was again infected with *P. vivax* and the infection was allowed to continue till he had 8 well-marked rigors (case IV). Nicol (Nicol & James, 1933) remarked that on analysing the cases at Horton, it was found that there was a tendency to better results in those cases which had more than 6–8 peaks of fever.

Contrary to the findings of Van Rooyen and Pile we have noticed that infection with *P. knowlesi* is very readily amenable to atebrin (cases IV and V).

Summary and Conclusions.

1. *P. knowlesi* seems to be suitable for the treatment of aged and debilitated individuals owing to the mild nature of the symptoms it produces.

2. As Wassermann positive blood is not suitable for all conditions in which malaria therapy is indicated, the blood of the monkey which is free from syphilitic infection can be safely used for the purpose.

3. An outstanding advantage is the readiness with which the monkey parasite can be maintained in the laboratory by passage from monkey to monkey.

4. A fairly large percentage of cases of infection with *P. knowlesi* has a tendency to spontaneous recovery—the parasites disappearing from the blood and the fever subsiding in a week or so. This mildness of symptoms renders *P. knowlesi* not quite as an effective therapeutic agent as human malaria.

5. Previous infection with human malaria produces a marked degree of tolerance to infection with *P. knowlesi*. Thus in localities where malaria is prevalent *P. knowlesi* does not appear suitable for therapeutic purposes.

6. An infection with *P. knowlesi* does not protect against human malaria.

7. When it is desired to give more than one course of fever to a patient, monkey malaria is of no use, inasmuch as one attack with this parasite seems to confer absolute immunity against a homologous strain of the same parasite.

8. In human infection with *P. knowlesi*, like quartan malaria, the fever may be pretty high even when the parasites are scanty.

9. Monkey malaria in human subjects can be very easily controlled by atebrin and quinine.

10. Neither form of malaria (of human or of monkey origin) seemed to produce any significant beneficial effect on the patient's nerve conditions.

References.

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