

7. Title: P. falciparum in Lower Primates (Monkeys and Tree Shrews)

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Objective: To develop an inexpensive and readily available system for use in laboratory studies of human malaria, particularly for evaluation of chemoprophylactics and chemotherapeutics.

Description: As a result of the success in establishing strains of P. falciparum in gibbons, it was decided to attempt subpassage from gibbons to several species of macaque monkey which are plentiful in this area and into tree shrews (Tupaia glis).

This report describes the successful infection of splenectomized macaque monkeys with P. falciparum both by subpassage from the gibbon (Hylobates lar) and by direct inoculation of human blood into monkeys.

All animals were studied before and after splenectomy for the presence of pre-existent malaria infection. All were negative but as a further precaution all animals were given a therapeutic course of chloroquine and primaquine after splenectomy.

In the first attempt, approximately 2.5 ml of heparinized blood from a Thai woman with P. falciparum infection was injected intravenously into a splenectomized gibbon. At six weeks when the density of parasites in the gibbon's blood was about 50,000/mm<sup>3</sup>, 2.5 ml of blood was withdrawn and injected intravenously into a second gibbon. Six weeks later when the parasitemia of this second gibbon was 4,000/mm<sup>3</sup>, 1.5 ml of heparinized blood from this gibbon was injected intravenously into each of three splenectomized monkeys; one Rhesus (Macaca mulatta siamica), one Pigtail (M. nemestrina), one Cynomolgus (M. iris). The major crossmatch (donor cells with recipient serum) showed gross agglutination and clumping in each instance. There was no evidence of adverse clinical effect on the monkey. Blood smears were examined daily for the presence of malarial parasites over a thirty-two week period. The Rhesus, Pigtail and Cynomolgus monkeys became positive on days 14, 6 and 10 respectively and detectable parasitemia persisted for 18, 19 and 16 weeks. Peak parasitemias were 850, 2420 and 1750 per cubic millimeter on days 30, 48 and 29. Generally the parasitemias, although detectable, were of very low magnitude. Attempts with first, second and third gibbon passage material were all successful in Cynomolgus monkeys but gave no suggestion that these levels of passage affected adaption of the parasite to an hetero logous host.

Direct human to monkey transfer of P. falciparum was attempted with Pigtail and Cynomolgus monkeys. In the first trial, heparinized blood from an infected Thai woman was injected intravenously into a gibbon, a Pigtail monkey and a Cynomolgus monkey. The gibbon's infection was patent on day 7, the Pigtail's on day 9 and the Cynomolgus on day 10. The peak of the gibbon's parasitemia (43, 640/mm<sup>3</sup>) occurred on day 69 and the parasitemia was persistent at detectable levels for seventeen weeks. The Pigtail had a peak of 220/mm<sup>3</sup> on day 23 and 35. Parasitemia was consistently detectable for about seven weeks and intermittently thereafter. The Cynomolgus had a peak of 450 on day 10 with persistent parasitemia for the nineteen weeks it was watched thereafter. In a second trial, four Cynomolgus monkeys and a gibbon were inoculated with blood from an infected Thai male. The prepatent period in the gibbon was 4 days and in the monkeys 6, 7, 7 days. The fourth monkey was still negative at 35 days.

In no instance was there any sign or symptom of disease in the monkeys. No gametocytes have been seen in the monkeys although immature gametocytes have been seen in the gibbons.

As a result of the successful colonization of the tree shrew (Tupaia glis) reported elsewhere under the heading of Laboratory Animal Development, it was decided that this would be an excellent animal for malaria studies if it were susceptible to human malarias. Consequently, two splenectomized tree shrews were inoculated with third gibbon passage P. falciparum. The inocula were in excess of 80,000 trophozoites per animal. Both animals became positive in eight days. One remained positive for sixteen weeks, the other for twenty-two weeks (but with a period of two weeks in which parasitemias were not detected). Parasitemia was of very low levels, the peak being approximately 150 per cmm. Two animals inoculated with P. falciparum which had been passed twice in gibbons and once in monkeys became positive on days 8 and 18 respectively. These animals received an inoculum of about 4,000 parasites each. Parasitemias persisted for 15 and 19 weeks and reached a maximum of 220 per cmm. Two animals inoculated with infected human blood (approximately 4,000 parasites) became positive in eight days and persisted for eight weeks with a high of 68 parasites per cmm. No gametocytes have been seen. Inoculation from tree shrews back into gibbons has been successful but parasitemia was of low magnitude.

The parasites seen in the monkeys and tree shrews are extremely small. No larger forms have been seen.

Summary: The Cynomolgus or crab-eating macaque is a prolific and relatively inexpensive monkey which is easily adapted to life in a laboratory environment and which is more tractable than the Rhesus or Pigtail. For these reasons, this would seem to be the animal of choice for future work. Although parasite densities have been extremely low in early passages, the persistency of parasitemia for 17-18 weeks would seem to indicate that the parasite can adapt to this host. The potential of these readily available, comparatively inexpensive animals as hosts for P. falciparum in chemotherapeutic trials urges further work to increase the density of parasitemia and, if possible, to produce disease symptoms.