# Transmission of mammalian malaria using immature exoerythrocytic schizonts

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*Summary.* A method has been developed for transmitting infection of mammalian malaria by inoculation of exoerythrocytic schizonts. The possibility that infection has been induced by other life cycle stages has been experimentally excluded.

Prophylaxis against human malaria, whether by drugs[1] or vaccination[2], is aimed at halting the first multiplying stage in man, the exoerythrocytic schizont (EES). Elimination of this stage would prevent the subsequent disease associated with parasite invasion of red blood cells. However, aside from morphology, surprisingly little is known about the EES. This ignorance is due in part to the difficulty associated with experimental manipulation of living EES outside of their original hosts. Indeed, Shortt and Garnham[3] have argued that the inability to infect other animals with inoculated liver EES was one of the reasons why the mammalian EES cycle remained undiscovered for so long. In spite of these technical difficulties, the development of a practical method for transmitting mammalian malaria by the inoculation of developing hepatic EES is highly desirable. Such a system would be useful for assessing the causal prophylactic activity of candidate antimalarial compounds and would provide a means of manipulating mammalian malaria EES for numerous basic studies which have been thus far impossible.

We have developed a simple method for transmitting infection of rodent malaria (Plasmodium berghei) by inoculation of developing EES. To ensure the validity of our conclusion, we have experimentally excluded the possibility that infection has been induced by other life cycle stages of the parasite.

Rats used as donors of hepatic EES were injected i.v. with $1–2 \times 10^6$ salivary gland sporozoites of the NK strain of P. berghei berghei. The minimum incubation time for this strain of malaria in white rats is 41.3 h (Foley and Vanderberg, unpublished data). The incubation time is that period between injection of sporozoites and the first appearance of merozoites in the blood. Thus, EES which are 21–28 h old have completed only $^1/_2$–$^2/_3$ of their normal minimum development time in the donor livers. EES of this age do not contain merozoites recognizable by light microscopy[4]. The donors were anesthetized 21–28 h after sporozoite injection and their livers rapidly removed and carefully sliced into fragments 0.5–1.0 mm in greatest dimension. These fragments were then implanted i.p. in anesthetized recipient rats or mice (see table). The blood of these recipients was then examined periodically for 3 weeks for blood forms of P. berghei. We found that liver containing immature EES is infective: 39 out of 44 recipient rats and 14 out of 19 recipient mice developed patent blood infections (table).

We did several control experiments to ensure that the infection which developed in the liver EES recipients was due to EES. To guarantee the absence of precocious blood merozoites, we inoculated heart blood from several liver EES donor rats into merozoite susceptible mice at the time when EES transplantations were done. The recipients of this blood never became infected.

To experimentally exclude the possibility that infectivity was due to latent sporozoites unintentionally transferred with the liver fragments, we used sporozoite-immune mice as the liver recipients in several experiments (table). Mice immunized with irradiated sporozoites are completely protected specifically against infection with viable sporozoites[5]. However, these mice will readily develop patent infections upon inoculation with merozoite-infected erythrocytes[6]. We reasoned that if the sporozoite-immune mice were susceptible to infection with liver containing EES, we could assume that infection was not due to latent sporozoites carried to the recipients in the liver fragments. We found that sporozoite-immune mice are as susceptible to infection with hepatic EES as are the non-immunized mice with which they were compared (table). However, our interpretation of these results is dependent upon the supposition that immunity to sporozoites in these immunized mice remains intact throughout our experimental

Transmission of Plasmodium berghei infection by inoculation of developing exoerythrocytic schizonts (EES)

| EES donors Number | Type | EES recipients Number | Number and percent (%) developing parasitemia |
|---|---|---|---|
| 27 | Sprague-Dawley female rats 35–85 g | 44 | 39 (88.6) |
| | A/J female mice (non-immunized) | 9 | 7 (77.8) |
| 2 | A/J female mice (sporozoite-immune) | 10 | 7 (70.0) |

All exoerythrocytic schizont (EES) donors were female Sprague-Dawley rats weighing 35–85 g which had been injected 21–28 h previously with $1–2 \times 10^6$ sporozoites. Each rat or mouse recipient was inoculated i.p. with 150–250 mg of liver fragments from a single donor. In some cases, multiple recipients were used for the liver from each donor. The number of recipients represents the number of animals surviving 3 weeks after EES inoculation. Recipients developing parasitemias are those which reached a patent blood infection within 3 weeks following EES inoculation. The immunized group of recipient mice was immunized with irradiated sporozoites by the method of Nussenzweig et al.[5].

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3 H. E. Shortt and P. C. C. Garnham, Trans. r. Soc. trop. Med. Hyg. *41*, 785 (1948).
4 M. Yoeli, R. S. Upmanis, J. Vanderberg and H. Most, Milit. Med. *131*, 900 (1966).
5 R. Nussenzweig, J. Vanderberg and H. Most, Milit. Med. *134*, 1176 (1969).
6 R. S. Nussenzweig, J. P. Vanderberg, H. Most and C. Orton, Nature *222*, 488 (1969).

procedure. To test the effect of the experimental manipulations on the protective immunity of immunized mice to sporozoites, a second variety of control experiment was done. In this experiment 2 sporozoite-immune and 2 non-immunized mice received 150–250 mg of noninfected liver fragments IP and 1 h later received 50,000 sporozoites i.v. The non-immunized mice in this experiment subsequently developed parasitemias while the sporozoite-immune mice remained uninfected. It is therefore clear that the manipulations involved in this type of transplantation are not sufficient to destroy the specific immunity to sporozoites in these mice.

In the single report of apparently successful transmission of mammalian liver EES, Rossan et al.[7] transplanted liver fragments containing developing EES of Plasmodium cynomolgi from sporozoite infected monkeys to the peritoneums of recipient monkeys at various times during the incubation period of the EES. These liver transplants were only occasionally infective to the recipient monkeys from the 4th to the 6th day post-injection of sporozoites into the donors. However, although the blood of the liver donors contained no infective parasites at these times, it was not technically possible for Rossan et al.[7] to eliminate the possibility of transfer of sporozoites with the liver fragments.

Because we can now totally immunize mice against infection with sporozoites, we have been able to experimentally exclude the possibility that infectivity of inoculated liver is caused by transferred sporozoites. By the results which we have obtained in these preliminary experiments we have demonstrated that it is possible to transmit rodent malaria by transplantation of developing EES from rats to both rats and mice. This procedure may be performed rapidly and provides a practical percentage of EES infected animals for applied experimental studies on this little understood stage of the mammalian malaria parasite. Because we have demonstrated experimentally that neither precocious merozoites nor latent sporozoites were responsible for the transmission of the plasmodium infection, we must conclude that the infectivity was due to viable immature EES in the transplanted liver fragments. We further conclude that at least some of these immature EES underwent continued development to mature merozoites and subsequently successfully invaded the blood streams of their recipient hosts.

7 R. N. Rossan, K. F. Fisher, R. D. Greenland, C. S. Genther and L. H. Schmidt, Trans. R. Soc. trop. Med. Hyg. *58*, 159 (1964).

## Effects of bilateral lesions of the nuclei habenulae on plasma thyroxine levels in Japanese quail

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*Summary.* Electrolytic bilateral lesions of the nuclei habenulae were made in male, adult photostimulated quail. Habenular destruction led to a marked decrease in the plasma thyroxine level (40%), whereas sham operated birds did not differ from controls. This result appears to be somewhat different from those obtained in mammals and the mechanisms of habenular-thyroid interrelationships are unknown.

The functional importance of the habenular complex is not completely understood. It was suggested[2–4] that the nuclei habenulae may be involved in thyroid regulation, in mammals. Various experimental data appear to indicate that habenular nuclei and surrounding regions exert an inhibitory effect upon the whole pituitary-thyroid system. However, it was found in other studies that bilateral ablation of the habenula does not interfere with thyroid activity[5,6]. On the other hand, bilateral habenular lesions were found to impair the pineal inhibitory response to a flash of light in unanaesthetized resting quail[7]. It was, therefore, interesting to investigate the habenular involvement in neuroendocrine regulation in birds. The aim of the present study was to explore the thyroid activity in quail, after destruction of the habenular nuclei, by determining the plasma thyroxine level. Adrenal cortical investigations in the same lesioned birds have been discussed elsewhere[8].

*Material and methods.* 20 adult male quail were used in this experiment. Birds were reared under controlled temperature (26 °C) and artificial lighting (18 h light: 6 h dark; light on: 06.00 h, light off: 24.00 h). Medial and lateral nuclei were located stereotaxically[9] under Equi-Thesin (Jensen Salsbery) anaesthesia. Lesions were produced by passing a direct current (250 μA for 25 sec) through a platinum electrode, 0.14 mm in diameter. In order to destroy completely the median and lateral habenula, it was necessary to place 2 lesions on either side, because these nuclei constitute a rather flat and thin structure. Stereotaxic coordinates are given in the table. After surgery, birds were left at rest in the same environmental conditions. Blood was collected (between 09.00 h and 10.00 h, to avoid any circadian interference) 4 weeks after lesioning. Upon completion of the experiments, the head was perfused with saline followed by 10% formalin-saline. The brain was subsequently frozen and serially cut in 40 μm sections for staining by the Kluver-Barrera technique[10]. Body weight was noted on the day of surgery and at autopsy. Plasma thyroxine levels were determined by the competitive protein binding radioassay developed in birds by Astier et al.[11].

Lesioned birds were compared to intact (no treatment) and sham-operated quail. Sham-operation was performed by fixing the head in the stereotaxic frame, trepanning the skull and lowering the electrode to the habenular stereotaxic coordinates without delivering any current through the electrode.

*Results and discussion.* Results are shown in the table. In intact and sham-operated animals, plasma thyroxine level was found to be quite similar to the values reported in photostimulated male quail by Peczély, (unpublished results: from 3.3 to 4.4 ng/ml vs 4.1 ng/ml in present