DEMONSTRATION OF EXOERYTHROCYTIC STAGES OF PLASMODIUM VIVAX IN SAIMIRI SCIUREUS*

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We have continued studies on the exoerythrocytic development of the Achiote strain of *Plasmodium vivax* in Panamanian monkeys (BAERG et al., 1974). Two unaltered adult *Saimiri sciureus*, free of naturally acquired malaria, were inoculated with sporozoite suspensions intrahepatically (monkey 7005) and intrahepatically plus intraperitoneally (monkey 6965). The inocula were derived respectively from 26 and 46 *Anopheles albimanus* which had fed on *Aotus trivirgatus* donors. Liver biopsies were taken from the sites of inoculation and 5 μ histological sections were stained with Colophonium-Giemsa.

As shown in the Table, the exoerythrocytic (EE) phase was found in 7-, 9- and 10-day biopsics. An infection was initiated in 6965 after a prepatent period of 12 days, whereas 7005 was killed at the time of the second biopsy, before a parasitaemia could be established; the EE forms in the latter animal were more numerous in relation to the number of tissue sections examined.

At 7 days, individual schizonts appeared on from 1 to 4 serial sections. The mean longest cross-section diameter for the bodies was $23\cdot4\,\mu$. Minimum and maximum diameters ranged from $18\cdot8\times18\cdot8\,\mu$ for the smallest form to $23\cdot8\times28\cdot8\,\mu$ for the largest. Some shrinkage occurred in the majority of the parasites. They were circular to ovoid, and in all instances contained deeply-stained, large, irregularly-shaped or rounded nuclei, numbering from 5 to 12 among those measured. The cytoplasm was loose to compact, usually with several small vacuoles, and cleft formation was seen.

At 9 days, the mean longest diameter was 36-1 μ , significantly larger than for the earlier biopsy. Two of the 8 bodies appeared on as many as 8 sections. The smallest measured $13.8 \times 15.0~\mu$ and the largest $37.5 \times 50.0~\mu$. Considerable variation in morphology was observed. Although most of the parasites were ovoid, others were irregular with incipient lobing. Again, shrinkage was common, being moderate to severe. Seven bodies apparently were developing normally. While I of these showed complete merozoite formation and was in the initial state of rupture, the remainder were characterized by finely-divided nuclei, with well stained, compact, granular cytoplasm. The growth of the eighth schizont, in contrast, was similar to that seen at 7 days.

The 10-day stages were readily found, and most were larger (a mean of $47.8\,\mu$ for bodies maintaining structural integrity) than those from the 9-day biopsy. Parasites occurred on 4 to 9 tissue sections, with measurements ranging from $32.5\times38.8\,\mu$ to $30.0\times58.8\,\mu$. Moderate shrinkage occurred in all, and most were ovoid in shape with limited lobing. Approximately 1 of every 8 bodies had ruptured, with release of merozoites into the surrounding tissue. Among those still intact, discrete merozoite formation also was evidenced. The schizonts were dense, with uniformly dispersed material, often more heavily stained on one side. In some instances, there were deeply stained aggregations of merozoites. From 1 to 5 rather large vacuoles, with a maximum diameter of $10.0\,\mu$, were present in half the bodies.

TABLE I. Results of studies with Plasmodium vivax excerythrocytic stages in Saimiri hosts.

Biopsy day	Monkey no.	Tissue examination		Maximum diameter of bodies	
		No. sections	Total no. bodies	No. measured	Mean (range) -p
7	7005	76	14	10	23-4 (18-8-28-8)
9	6965	324	8	8	36-1 (15-0-50-0)
10	7005	70	38	10	47-8 (38-8-58-8)

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In the 10-day biopsy there were 3 unusual EE forms, characterized by 2 or 3 large (6·3 to $23\cdot8\,\mu$) round to ovoid masses which occupied most of the cytoplasm. These areas were opaque, dark violet in colour and they contained numerous particles somewhat larger than the normally developing merozoites. Under light microscope magnification of $1,000\times$ a membrane appeared to surround each mass, with shrinkage from the cytoplasm. We expect to study this atypical morphology in detail.

Our earlier findings (Young et al., 1971) showed that S. sciureus is susceptible to trophozoite and sporozoite induced infection with the Achiote strain of P. vivax. The present report indicates the suitability

of Saimiri for EE stage investigations.

References

BAERG, D. C., ROSSAN, R. N., & YOUNG, M. D. (1974). Am. J. trop. Med. Hyg., 23, 710 YOUNG, M. D., BAERG, D. C., & ROSSAN, R. N. (1971). Trans. R. Soc. trop. Med. Hyg., 65, 835.