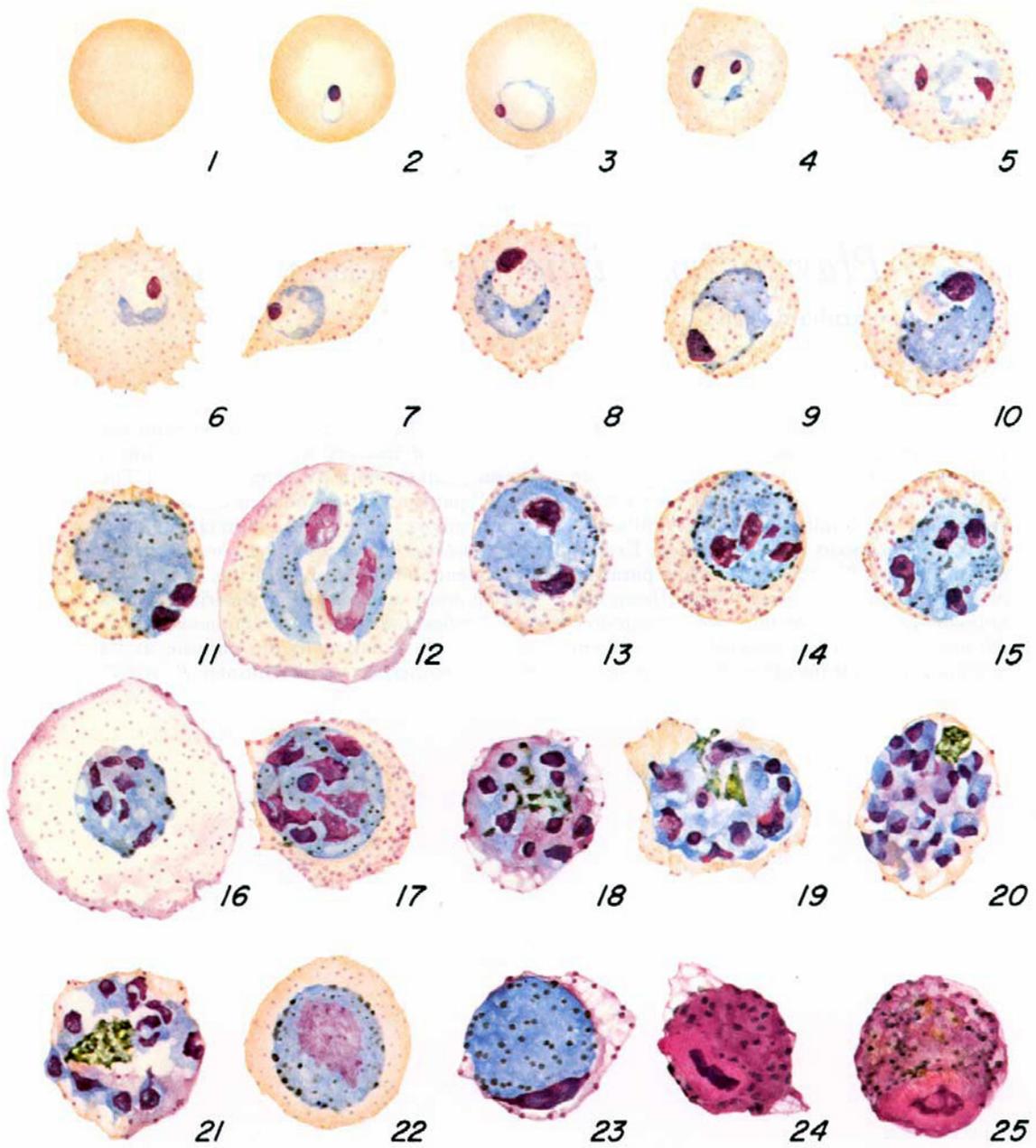


Plasmodium simiovale Dissanaïke, Nelson, and
Garnham, 1965

DURING the course of an extensive survey of simian malaria in Ceylon, Dissanaïke obtained blood from two toque monkeys, *Macaca sinica*, shot near the Mi-Oya river, near the 7-mile post on the Puttalam-Anuradhapura road in N. W. Ceylon. Examination of blood films did not disclose parasites of malaria, but when heart blood from the animals was inoculated into two parasite-free *M. sinica*, each became infected. One of them exhibited a double infection: *Plasmodium shorti*

and a "new" parasite. When blood from the dual-infected monkey was inoculated into a rhesus monkey, both parasites appeared. The "new" parasite was obtained as a pure line in rhesus monkeys by passage through mosquitoes, *Anopheles atroparvus*, at the London School of Hygiene and Tropical Medicine. Dissanaïke, Nelson and Garnham (1965) described it as a new species under the name *Plasmodium simiovale* to call attention to the parasite as "a simian counterpart of the human *P. ovale*."



0 10μ

PLASMODIUM SIMIOVALE

B. H. Nicholson

Cycle in the Blood

PLATE XXX

The first parasites to appear in the peripheral blood are delicate ring forms with prominent nuclei. As the parasite grows, the ring form may display an accessory chromatin dot, oftentimes within the vacuole, and occupies a fair portion of the host cell which has increased in size (Fig. 3). With further growth, the erythrocyte may become distorted. The stippling, at first small and delicate, becomes granular and prominent. In heavy infections, especially, multiple invasion of the host cell is common (Figs. 5, 12). The vacuole of the early stages becomes much larger and may occupy a third or more of the parasite. The cytoplasm of the young non-amoeboid parasite becomes more dense, sometimes enclosing small vacuoles. Dark granular pigment is scattered in the cytoplasm. The nucleus is large and stains a dark red. By the end of the trophozoite stage, the large vacuole has disappeared and the parasites begin nuclear division. As schizogony proceeds, small cleft-like or circular vacuoles appear in the cytoplasm (Figs. 13, 14); the pigment remains granular but definite. Some of the host cells are greatly enlarged and display an eosinophilic ring. The cytoplasm is discolored but the enclosed parasite appears normal (Fig. 16). Schizogony continues, finally producing 12 to 16 merozoites. The enlarged host cell is generally distorted and the parasite tends to be oval or roughly circular. Vacuoles are in evidence; the pigment granules come together in loose aggregates and finally form a solid greenish-yellow mass. During early schizogony, the stippling in the host cell remains prominent, but toward the end, it collects toward the periphery; the cell is discolored and generally distorted (Figs. 13-21).

Spherical gametocytes appear early during the initial parasitemia. The macrogametocytes far outnumber microgametocytes. The growing female parasites are difficult to distinguish from the mature trophozoites because the latter often fill the corpuscle and their pigment too is scattered in the cytoplasm. The young adult distaff parasites are circular and occupy a fair portion of the enlarged host cell which shows delicate diffuse stippling (Fig. 22). The fully mature macrogametocyte is compact, stains a deep blue and has scattered granular dark pigment. The nucleus is marginal, elongated, and stains a deep red. The parasite may not fill the depleted host cell, which is distorted; stippling is prominent and located near the periphery (Fig. 23). The microgametocyte stains red to pink. In the oldest forms, the pigment is granular, and scattered in the cytoplasm. The nucleus which occupies about a third of the parasite is without pigment and embraces a darker staining bar- or skein-like area. The adult parasite fills the host cell (Figs. 24, 25).

The asexual cycle occupies 48 hours.

Sporogonic Cycle

PLATE XXXI

Mosquito infections were reported by Dissanaïke *et al* (1965) in *Anopheles atroparvus* and *A. stephensi*. In the former, oocysts containing dark discrete grains of pigment, arranged in irregular lines, measured 23 to 35 μ after 7 days of extrinsic incubation at 26° C. On the 9th day, oocysts measured 35 to 45 μ . At this time, the pigment grains were concentrated over a small area or were obscure. By the 11th day, oocysts measured 40 to 50 μ . Sporozoites appeared in the salivary glands 13 days after feeding. In *A. stephensi*, one oocyst was seen 5 days after feeding. This oocyst measured 15 μ in

PLATE XXX.—*Plasmodium simiovale*.

Fig. 1. Normal red cell.

Figs. 2-4. Young trophozoites.

Figs. 5-9. Growing trophozoites.

Figs. 10, 12. Nearly mature and mature trophozoites.

Figs. 11, 13-18. Developing schizonts.

Figs. 19-21. Mature schizonts.

Figs. 22-23. Young adult and mature macrogametocytes.

Figs. 24-25. Mature microgametocytes.

diameter and the pigment was in lines on the periphery.

In our studies, oocyst development was studied in *A. b. balabacensis*, *A. maculatus*, *A. freeborni*, and *A. stephensi* mosquitoes incubated at 25° C (Table 25).

In *A. b. balabacensis*, at 4 days, the oocysts ranged in diameter from 9 to 12 μ with a mean of 10 μ . The oocysts continued to grow so that

by day 12, their size ranged from 26 to 83 μ , with a mean of 67 μ . Sporozoites were present in the salivary glands on day 13. In the *A. maculatus*, although the oocyst diameters were within the range of those seen in the *A. b. balabacensis*, the mean diameters, after 10 or more days of extrinsic incubation, were smaller. Sporozoites did not appear in the salivary glands until day 14. Although oocysts developed and

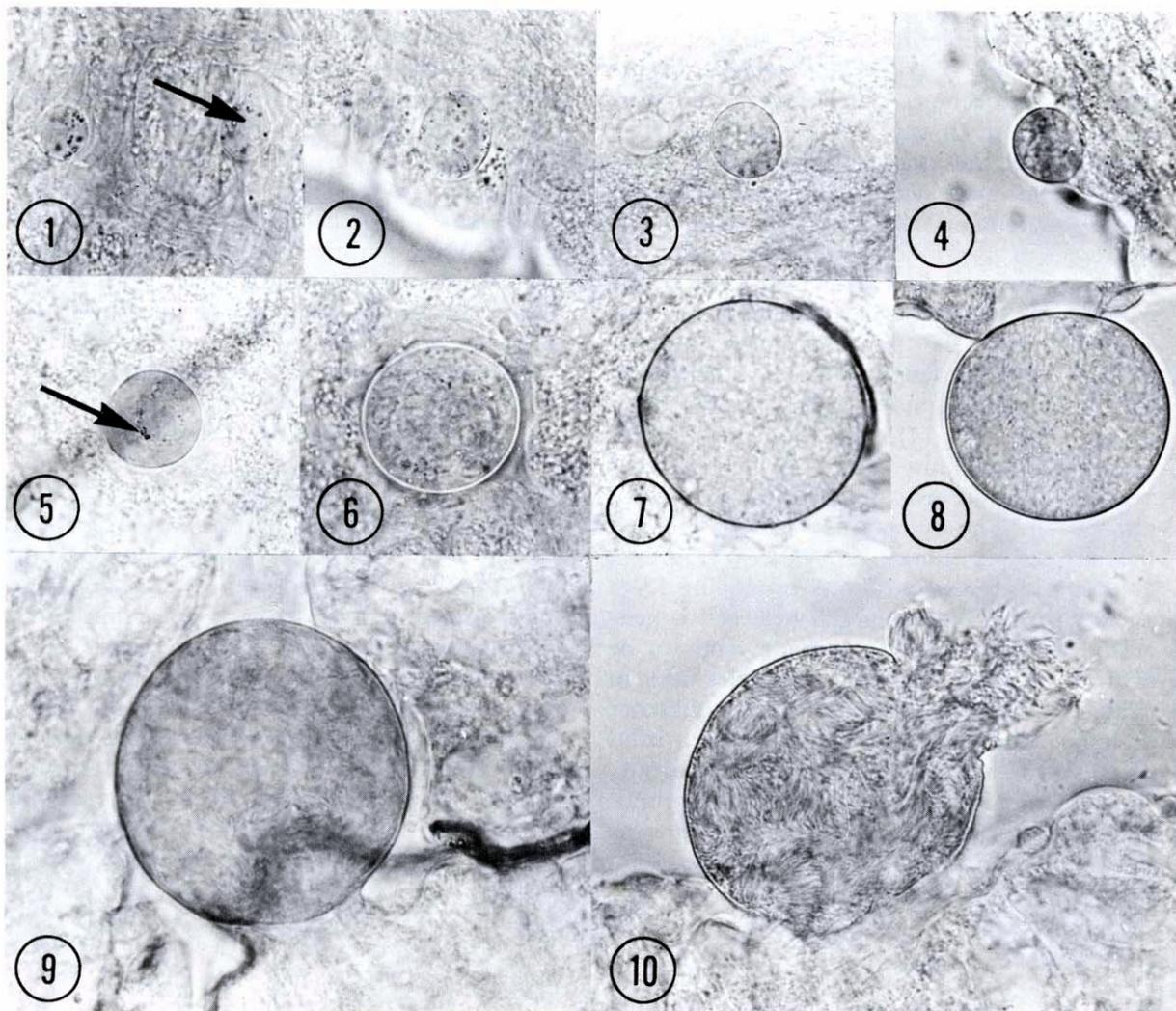


PLATE XXXI.—Developing oocysts of *Plasmodium simiovale* in *Anopheles b. balabacensis* mosquitoes. X 580 (Except Figs. 1 & 2).

Fig. 1. 4-day oocyst showing abundant scattered pigment.

X 740.

Fig. 2. 5-day oocyst showing scattered pigment. X 740.

Fig. 3. 6-day oocyst.

Fig. 4. 7-day oocyst.

Fig. 5. 8-day oocyst still showing pigment.

Fig. 6. 9-day oocyst.

Fig. 7. 10-day oocyst.

Fig. 8. 11-day oocyst.

Fig. 9. 12-day differentiating oocyst.

Fig. 10. Rupturing 12-day oocyst showing release of sporozoites.

TABLE 25.--Oocyst diameters of *Plasmodium simiovale* in *Anopheles b. balabacensis*, *A. maculatus*, *A. freeborni*, and *A. stephensi*.

Days after Infection	<i>A. b. balabacensis</i>			<i>A. maculatus</i>			<i>A. freeborni</i>			<i>A. stephensi</i>		
	No.	Range	Mean*	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
3												
4	25	9-12	10				29	7-13	8	2	8-13	11
5	53	11-15	13				44	8-13	11	21	12-15	13
6	66	12-19	16	115	13-24	19	120	12-22	17	23	11-19	16
7	116	13-26	19	168	12-30	22	189	13-33	23	55	12-26	21
8	143	17-39	27	65	15-41	28	167	15-42	28	4	24-30	26
9	111	17-54	39	124	18-51	33	132	17-48	35	8	24-51	41
10	131	20-63	42	111	22-65	40	114	26-57	41			
11	171	25-73	54†	93	24-63	45	135	20-77	53†			
12	110	26-83	67†	143	24-73	48†	179	25-87	58†	15	28-68	56†
13	126	37-92	67†‡	145	24-74	54†	129	32-90	67†	2	47-61	54†
14	93		67†‡	60	24-80	57†‡	125	26-97	67†			
15			67†‡				97	21-87	58†			
Totals	1145	9-92		1024	13-80		1575	7-97		130	8-68	

* Measurements expressed in microns.

† Oocyst differentiation.

‡ Sporozoites present in the salivary glands.

showed differentiation in the *A. freeborni* mosquitoes, infection of the salivary glands was much later and then only at a very low level. Measurement of oocysts in the *A. stephensi* mosquitoes gave diameters in the range of those seen in the *A. b. balabacensis*. An insufficient number of the *A. stephensi* mosquitoes survived to determine if sporozoites would or would not invade the salivary glands.

A comparison of the oocyst growth curves of *P. simiovale* and *P. cynomolgi* in *A. b. balabacensis* mosquitoes (Fig. 42) discloses that *P. simiovale* takes 3 days longer to complete its development than does *P. cynomolgi*. The mean oocyst diameter of *P. simiovale* on day 11 was approximately the same as that of *P. cynomolgi* on day 9. However, the former parasite develops to a maximum size slightly larger than does the *P. cynomolgi* parasite. A comparison of the oocyst growth curves between *P. simiovale* and *P. fieldi* (Fig. 43) indicates a very close

relationship between them. The *P. fieldi* parasite requires one day longer to complete its sporogonic cycle in *A. b. balabacensis* mosquitoes than does *P. simiovale*.

Garnham (1966) reported that sporozoites of *P. simiovale* in dried preparations are 12 to 14 μ in length. Although few sporozoites survived in *A. atroparvus*, transmission was obtained via the bites of infected mosquitoes of this species. The prepatent period in the recipient *M. mulatta* monkey was 24 days, which agrees with Dissanaïke *et al* (1965).

In our studies, transmission was obtained on two occasions. Dissected salivary glands from *A. b. balabacensis* were inoculated intra-hepatically into an intact *M. mulatta* monkey. The animal developed an infection with *P. simiovale* with a prepatent period of 11 days. Another monkey was bitten by infected *A. maculatus* mosquitoes. The prepatent period in this animal was 17 days. Attempts to transmit

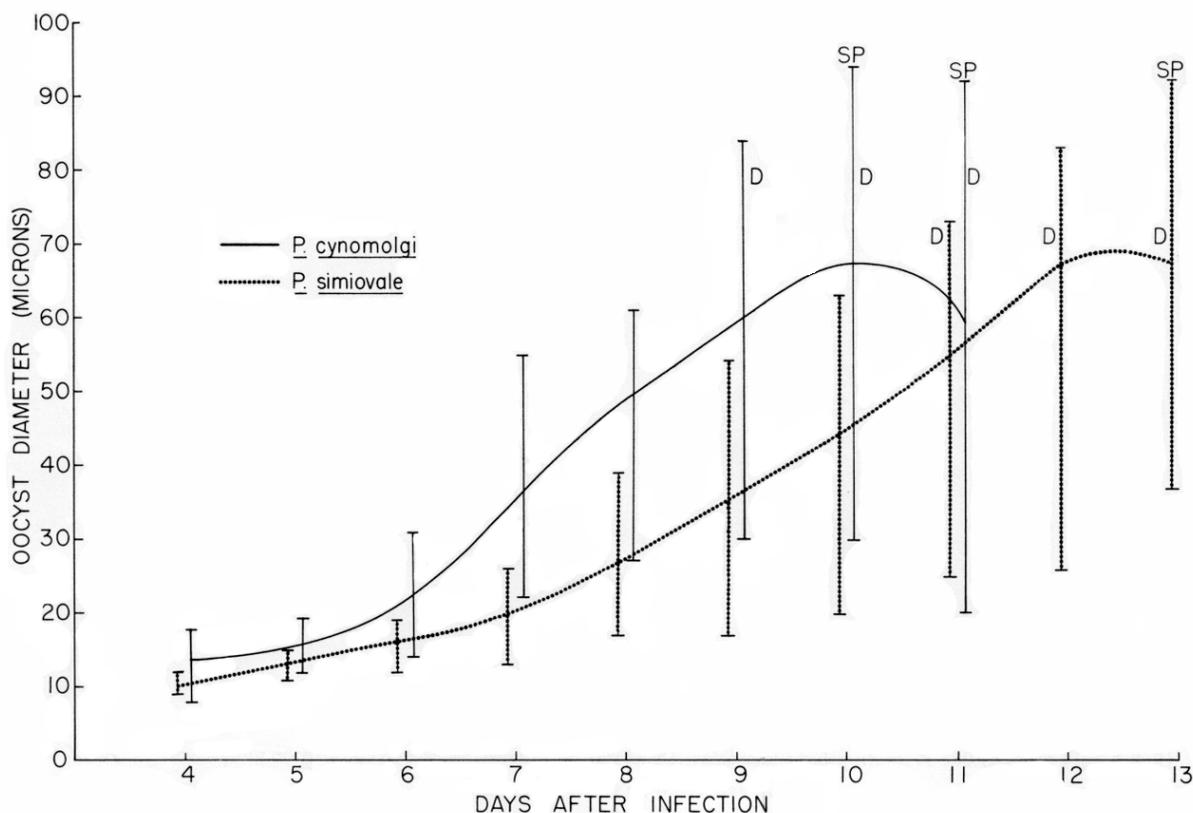


FIGURE 42.—Range in oocyst diameters and the mean oocyst diameter curves of *Plasmodium simiovale* and *P. cynomolgi* in *Anopheles b. balabacensis* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

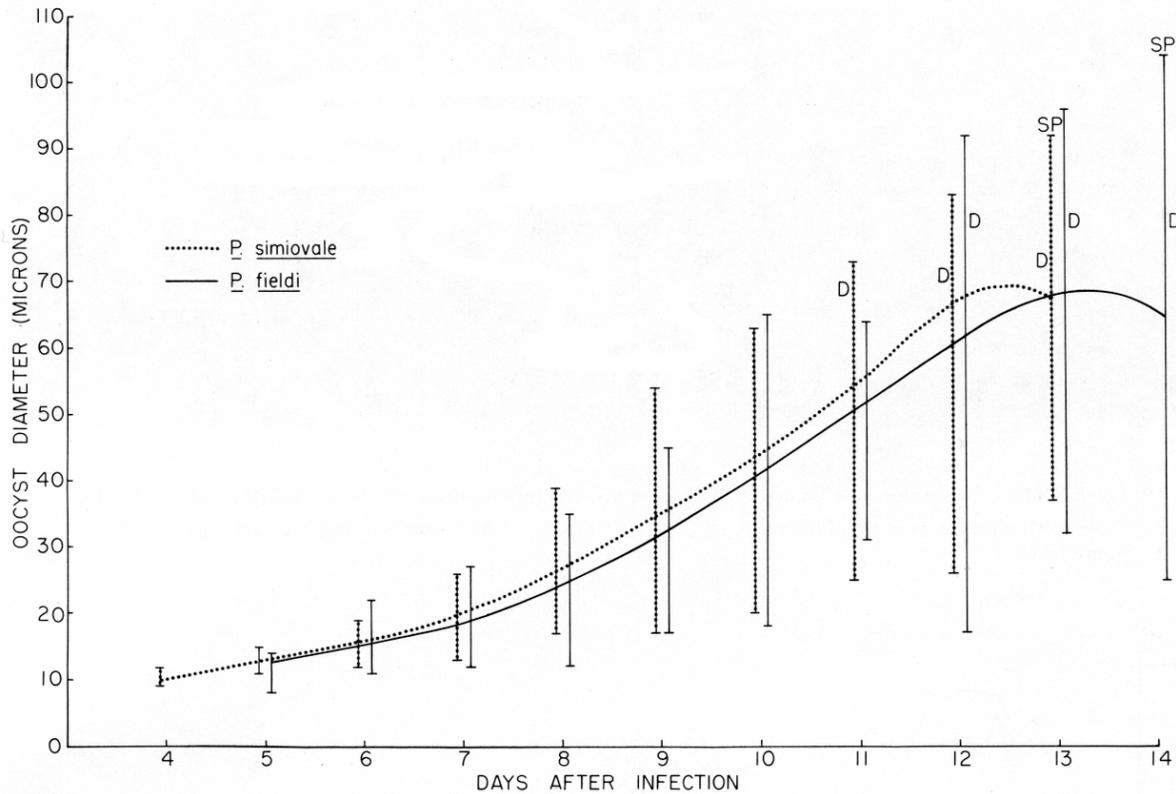


FIGURE 43.—Range in oocyst diameters and the mean oocyst diameter curves of *Plasmodium simiovale* and *P. fieldi* in *Anopheles b. balabacensis* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

the infection by the bites of infected *A. b. balabacensis* mosquitoes to two volunteers were unsuccessful.

Cycle in the Tissue PLATE XXXII

The exoerythrocytic stages of *Plasmodium simiovale* have been studied only recently. Thus far, we have seen only the 7-day forms (Figs. 1-3). Although smaller, these parasites are similar to the 8-day forms of *P. fieldi* described by Held *et al* (1967). Like the *P. fieldi*, the *P. simiovale* parasites contained numerous small flocculi and peripheral indentations (Fig. 2) described by Held *et al* (loc. cit.) as scalloping. The space occupied by 25 *P. simiovale* parasites ranged from 14 to 25 μ in width and from 16.5 to 29.5 μ in length. The average dimensions were 18.9 by 23.8 μ . Further studies will be needed before any

conclusions can be made concerning differences, if any, which may exist between the EE stages of this parasite, and the other primate malaras.

Course of Infection

In the normal host, *M. sinica*, the infection is mild (Dissanaike *et al*, 1965). In the *M. mulatta* monkey, the parasitemia never reaches a high level, even after splenectomy (Garnham, 1966). An examination of the parasitemia curves in the two monkeys infected in our laboratory by sporozoite inoculation confirms this observation (Fig. 44). The maximum parasite counts during the 60-day observation period were 21,300 and 31,740 per mm^3 . After the initial peak, the parasitemias quickly dropped to very low levels which were maintained for the remainder of the 60-day period of observation.

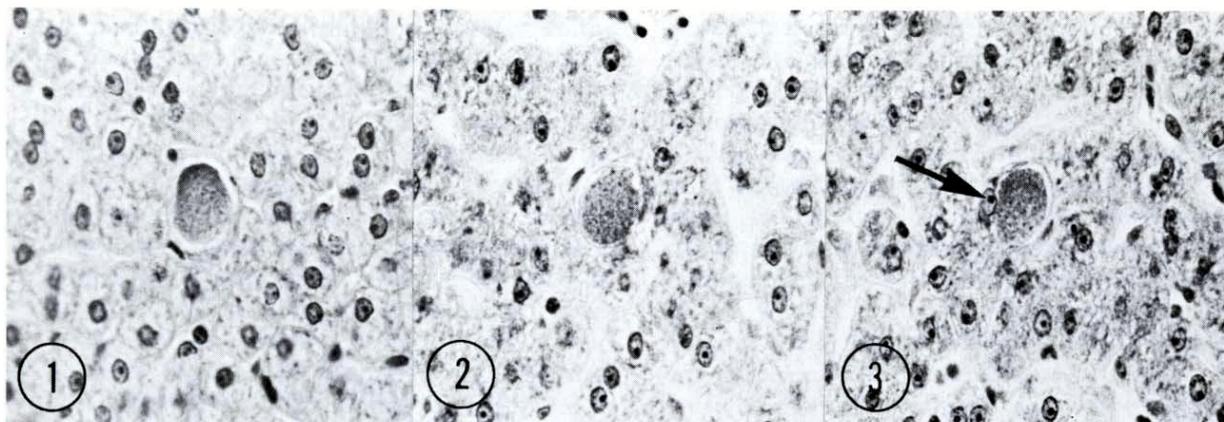


PLATE XXXII.—Exoerythrocytic bodies of *Plasmodium simiovale* in liver tissue of *Macaca mulatta* monkeys. X 580.

Fig. 1. 7-day body showing very small flocculi.

Fig. 2. 7-day body.

Fig. 3. 7-day body showing host cell nucleus.

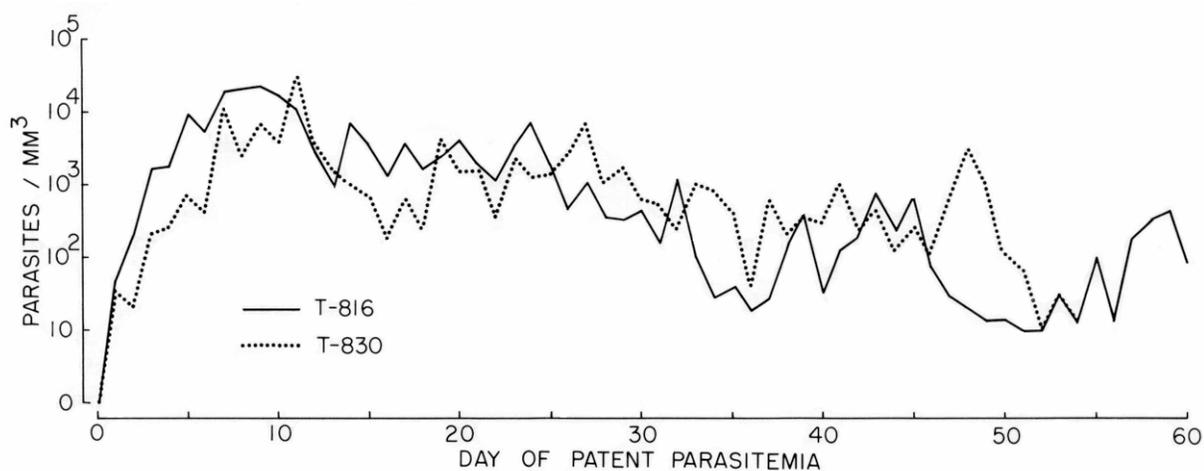


FIGURE 44.—Parasitemia curves of *Plasmodium simiovale* in two *Macaca mulatta* monkeys infected via the bites of infected mosquitoes.

Host Specificity

The natural host of *Plasmodium simiovale* is *M. sinica* (Dissanaike *et al.*, 1965). Experimentally, *M. mulatta* is also susceptible to infection either by blood- or sporozoite-inoculation.

The natural vector of this parasite is unknown. Dissanaike *et al.* (1965) reported the experimental infection of *A. stephensi* and *A. atroparvus*); the latter species being apparently more susceptible to the infection. We have infected *A. b. balabacensis*, *A. freeborni*, *A. maculatus*, *A. stephensi*, *A. atroparvus*, and *A.*

quadrimaculatus mosquitoes (Table 26). Only *A. b. balabacensis* and *A. maculatus* readily support development to the presence of sporozoites in the salivary glands. Because the morphology of the blood forms of *P. fieldi* and *P. simiovale* are similar, it was expected that these species would also show a similarity in their infectivity to different species of mosquitoes. When such a comparison was made, however, it was found that the average number of oocysts per gut in *A. b. balabacensis* was higher than in *A. freeborni* infected with *P. simiovale*. The reverse was true with *P. fieldi* (see Chapter 16). Because the *A. freeborni*

mosquitoes are not co-indigenous with either of these malaras, it might serve as the standard for comparison. The *A. b. balabacensis* are co-indigenous with *P. fieldi* and would be expected to demonstrate a high level of susceptibility to it. However, the higher ratio of susceptibility was to the simiovale parasite indicating a true difference between these 2 parasites. If *A. b. balabacensis* is used as the standard, as in Table 26, there is little difference between the relative susceptibility of these parasites to *A. maculatus* (100:11.3) for *P. simiovale* and (100:16.5) for *P. fieldi*. However, if the non-co-indigenous mosquito, *A. freeborni*, is used as the standard, then the difference in mosquito susceptibility is apparent. Thus it can be said that these parasites do exhibit differences in their infectivity to mosquitoes which emphasizes their specificity.

Immunity and Antigenic Relationships

Apparently, a prior infection with *P. fieldi* in a rhesus monkey confers no immunity against an infection with *P. simiovale*. In our hands, an animal was infected with *P. fieldi* and allowed to run a course of patent parasitemia for 61 days. The maximum parasite count was 18,300 per mm³. The infection was then eliminated by treatment with chloroquine. Seventy-eight days later, the monkey was inoculated with blood containing parasites of *P. simiovale*. The infection became patent and followed a course normal for a blood-induced infection with a maximum parasitemia of 12,000 per mm³ 11 days after inoculation.

TABLE 26.—Comparative infectivity of *Plasmodium simiovale* to *Anopheles b. balabacensis*, *A. freeborni*, *A. maculatus*, *A. stephensi*, *A. atroparvus*, and *A. quadrimaculatus*.

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
Bal						100
Bal : F-1	12	127	377	67.7	48.8	55.4
Bal : Mac	14	193	465	54.9	34.8	11.3
Bal : St-1	8	168	161	44.6	21.7	10.9
Bal : Atro	8	50	236	76.0	16.5	4.1
Bal : Q-1	15	235	417	50.2	6.2	1.4

* Bal = *Anopheles b. balabacensis*, F-1 = *A. freeborni*, Mac = *A. maculatus*, St-1 = *A. stephensi*, Atro = *A. atroparvus*, Q-1 = *A. quadrimaculatus*.

**GII = Gut Infection Index = average number of oocysts per 100 guts; the GII ratio is the relationship of the GII of *A. b. balabacensis* to another species where the GII of *A. b. balabacensis* = 100.

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