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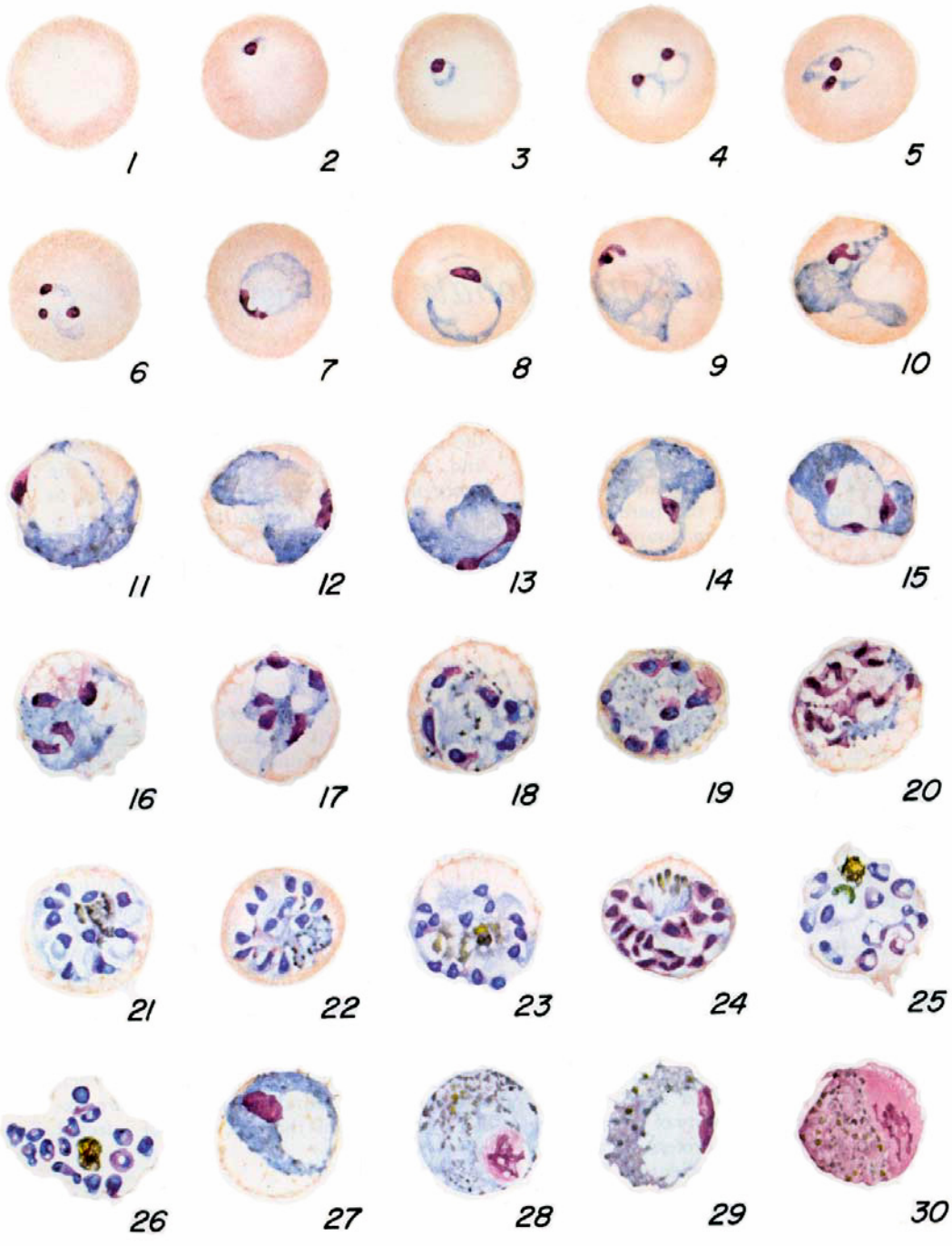
Plasmodium hylobati Rodhain, 1941

ON the 2nd of June 1939, Dr. Jerome Rodhain examined the blood of a gibbon, *Hylobates lensciscus* Geoff. from Java and in the blood, he found a few parasites of a malaria. He made a second examination five days later and saw that the parasites were more numerous. They then disappeared and he never saw them again. From the material collected on those two occasions, Rodhain described the parasite and named it *Plasmodium hylobati* in 1941. His description lacked completeness, in that he did not see microgamecocytes; but, it does allow for the recognition of the parasite. He predicted that the asexual cycle was quartan. According to Garnham (1966), Wenyon saw a similar parasite in other species of gibbons which had died in the London Zoo in 1946 and he, Garnham, saw it in films sent to him from Sarawak. This extra material was evidently used by Garnham in writing his description of the parasite.

The parasite has not been found in peninsular Malaysia or in Thailand and none of us had ever seen it. In fact, we despaired of locating material for a colored plate, and other studies, when a fortunate happening occurred. A mature male *Hylobates moloch* from North Borneo was received at the University of Singapore and found to be infected with a malaria tentatively identified as *P. hylobati.* Dr. Zaman, knowing the rarity of the parasite, sent the infected animal to Professor Garnham at the London School of Hygiene & Tropical Medicine so that further studies could be carried out. Professor Garnham also believed the parasite to be the long-sought *P. hylobati* and, anxious to have a demonstration of the exoerythrocytic stages, he sent the animal to us in Chamblee, Georgia, because we maintained a colony of *A. b. balabacensis* believed to be a potential vector. The gibbon was received at Chamblee in good health and carrying a low-grade infection of a malaria. Later, the animal was splenectomized; and with increased parasitemia, it was evident that the parasite was *P. hylobati.* Under those favorable conditions, mosquitoes were allowed to feed on the animal. At the same time, there was ample material for studying the periodicity of the asexual cycle and for transfer of parasitized blood to monkeys. Five species of mosquitoes were allowed to feed on the gibbon whereupon, its malaria was eliminated by treatment with chloroquine. When the test mosquitoes were found infected, they were dissected and their sporozoites inoculated intravenously into the original gibbon and, intrahepatically at laparotomy, into an owl monkey *(Aotus trivirgatus).* Following exposure to infection, liver biopsies were done on day 7 and 14 in the gibbon and on day 7 in the owl monkey. Exoerythrocytic parasites were found in each of the animals. Human volunteers were exposed to infection through bites of infected mosquitoes.



PLASMODIUM HYLOBATI

Cycle in the Blood
PLATE XI

The youngest erythrocytic parasites consist of a prominent nucleus and a small fragment of cytoplasm (Fig. 2). As the young parasite grows, the nuclear mass remains essentially unchanged while the cytoplasm increases both in volume and in the area of the host cell it occupies (Figs. 3-6). Multiple chromatin masses are not uncommon (Figs. 5, 6). Dual invasion of a single host cell has been observed (Fig. 4) but the condition is not considered characteristic of the parasite. The nucleus begins to change as the trophozoite continues to develop, stretching around the periphery of the vacuole or branching as it grows (Figs. 7-10). Pigment first appears as one or two accumulations of very fine, grayish-black granules causing areas of the cytoplasm to appear more gray than blue in the Romanowsky-stained young trophozoite (Fig. 10). The pigment becomes identifiable though not prominent in the young adult forms (Fig. 11). As the trophozoite approaches maturity, the cytoplasm thickens, takes a deeper stain, and the nucleus becomes more dense (Figs. 10-12). The pigment remains scarce and frequently hard to identify. There is no obvious host cell enlargement. Schüffner's stippling or other types of inclusions in the host cell have not been observed. Schizogony is unremarkable except that the host cell cytoplasm sometimes appears greatly depleted, leaving the parasite enmeshed in a network of fine cytoplasmic threads (Figs. 16-18, 20, 23). In some cases, the nuclear material is dominated by thick, tortuous threads or globs (Figs. 20, 24). As the schizont approaches maturity the pigment becomes more abundant and begins to accumulate into groups of moderately coarse, yellowish-black granules (Figs. 21, 23, 24). The mature schizont has from 12 to 20 merozoites, which may be arranged to form what Rodhain called a rosette; 14 to 16 is the most common number. When the merozoites are completely formed, the pigment assumes a single dense yellowish-black mass (Figs. 25, 26).

Young gametocytes are difficult to distinguish from young and mature trophozoites. However, the young microgametocyte does show some of the rare staining qualities of the cytoplasm fairly early (Fig. 29).

The mature macrogametocyte fills the host cell and displays smooth, uniformly staining blue cytoplasm. The pigment is in moderately coarse, randomly distributed granules. The nucleus is circular to oval, dense, and usually located at the periphery of the cell (Fig. 28).

The fully adult microgametocyte fills the host cell and the cytoplasm as well as the nucleus stains a bright rose-pink. The nucleus is large and diffuse with a dense central mass. Frequently, the nucleus and the cytoplasm stain a uniform pink. Separation is achieved because pigment granules are randomly distributed through the cytoplasm but they are absent from the nucleus (Fig. 30).

The asexual cycle in the blood occupies 48 hours.

Sporogonic Cycle
PLATE XII

The natural vector of this parasite is unknown. However, we were able to study the sporogonic cycle in five laboratory reared species of mosquitoes: *A. b. balabacensis* from

PLATE XI.—*Plasmodium hylobati.*

Fig. 1. Normal red cell.
Figs. 2-13. Developmental stages of the trophozoite.
Figs. 2-4. Rings stages.
Figs. 5, 6. Young trophozoites.
Figs. 7-9. Adolescent trophozoites.
Fig. 10. Young adult trophozoite.
Figs. 11, 12. Mature or adult trophozoites.
Fig. 13. Mature trophozoite with dividing nucleus.
Figs. 14-24. Schizogonic stages
Fig. 25. Submature schizont.
Fig. 26. Mature schizont.
Figs. 27-30. Gametocytes.
Fig. 27. Immature macrogametocyte.
Fig. 28. Mature macrogametocyte.
Fig. 29. Immature microgametocyte.
Fig. 30. Mature microgametocyte.

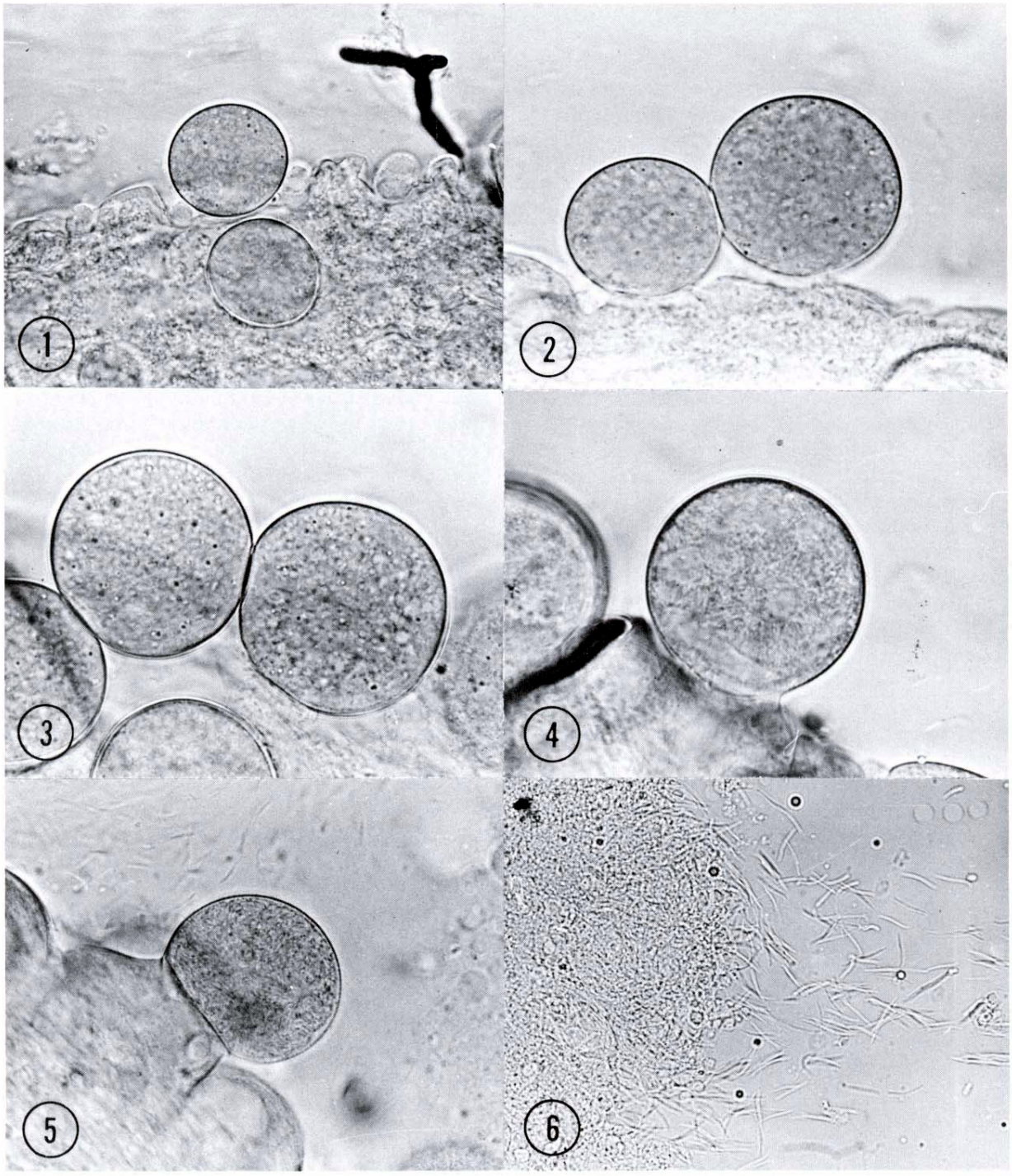


PLATE XII.—Developing oocysts and sporozoites of *Plasmodium hylobati* in *Anopheles b. balabacensis* mosquitoes. X 580.

Fig. 1. 8-day oocysts.
Fig. 2. 9-day oocysts.
Fig. 3. 10-day oocysts.
Fig. 4. 11-day oocyst showing differentiation.
Fig. 5. 12-day differentiated oocyst. Sporozoites free in fluid near gut.
Fig. 6. Sporozoites present near salivary gland tissue 12 days after feeding.

Thailand, *A. stephensi* from India, *A. maculatus* from Malaysia, *A. freeborni* and *A. quadrimaculatus* from the United States. Observations began 6 days after feeding and continued through day 13. The extrinsic incubation temperature was 25° C.

The results of the oocyst measurements are presented in Table 12. In *A. b. balabacensis,* on day 6, the mean oocyst diameter was 15 µ with a range of 11 to 20 µ. The oocysts continued to grow so that by day 12, the average size was 53 µ with a range of 30 to 70 µ. Sporozoites were first seen in the salivary glands on day 12.

The examination of the oocyst diameters in the other test mosquitoes indicated that the growth rate of the parasite was similar in each of the five species. Sporozoites were present in the salivary glands of *A. stephensi* on day 12 and in *A. freeborni* and *A. maculatus* on day 13. No sporozoites were found in the salivary glands of the *A. quadrimaculatus* through day 16.

In comparing the extrinsic development of *P. hylobati* in *A. b. balabacensis* with *P. cynomolgi,* from the rhesus monkey (Fig. 29), and *P. jefferyi,* from the gibbon (Fig. 30) one finds that the *P. cynomolgi* oocysts are much larger and that its sporozoites appear in the salivary glands two days earlier. *Plasmodium jefferyi* oocysts are considerably smaller than those of *P. hylobati* and its sporozoites appear in the glands one day later.

Comparison studies with *P. eylesi* could not be made because the growth studies with it were made at a different extrinsic incubation temperature.

The sporozoites of *P. hylobati* in *A. b. balabacensis* were infective because they initiated infection in a gibbon. The prepatent period was nine days.

Cycle in the Tissue
PLATE XIII

Sodeman *et al* (1971) ably described the tissue stages of *Plasmodium hylobati* found on day 7 and 14 in the gibbon and those seen on day 7 in the owl monkey.

The EE bodies were round to elliptical in shape with smooth edges. Some were retracted from their surrounding liver cell, probably as a result of fixation. The nuclei were round, although, infrequent bar-shapes were seen. The nuclei stained magenta, measured 0.5-1.5 µ in diameter, and were evenly distributed through the cytoplasm. The latter was granular in texture and stained pale blue. In many of the parasites irregular shaped, dark blue aggregates ("flocculi") were scattered diffusely through the cytoplasm but this feature was not universal. The flocculated material contained small holes. Parasitized liver cells were enlarged and their nuclei displaced peripherally. There was no nuclear enlargement of the parasitized liver cells; and, vacuolation of the cytoplasm was seen only occasionally. No mononuclear cell or

TABLE 12.--Oocyst diameters of *Plasmodium hylobati* in *Anopheles b. balabacensis, A. stephensi, A. freeborni, A. maculatus,* and *A. quadrimaculatus.*

Days after Infection	*A. b. balabacensis*			*A. stephensi*			*A. freeborni*			*A. maculatus*			*A. quadrimaculatus*		
	No.	Range*	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
6	150	11-20	15												
7	100	12-30	21	90	13-25	19	107	12-25	17	105	12-26	16	117	12-27	20
8	100	15-33	26	111	17-35	24	105	13-35	25	113	14-35	24	128	18-40	29
9	100	18-47	35	124	19-45	33	135	13-42	30	111	14-37	24	109	18-45	34
10	100	25-55	41†	100	27-55	42†	100	19-47	35	114	20-52	34	150	21-55	37
11	100	28-59	45†	100	24-65	39†	110	20-47	34	111	19-63	46†	100	21-61	41†
12	100	30-70	53†**	108	22-65	45†**	100	26-68	51† †**	114	20-64	45† †**	100	32-72	51† †
Totals	750	11-70	†**	633	13-25	†**	657	12-68	†**	668	12-64	†**	704	12-72	†

* Measurements expressed in microns; incubation temperature 25° C.
† Oocyst differentiation.
** Sporozoites present in the salivary glands.

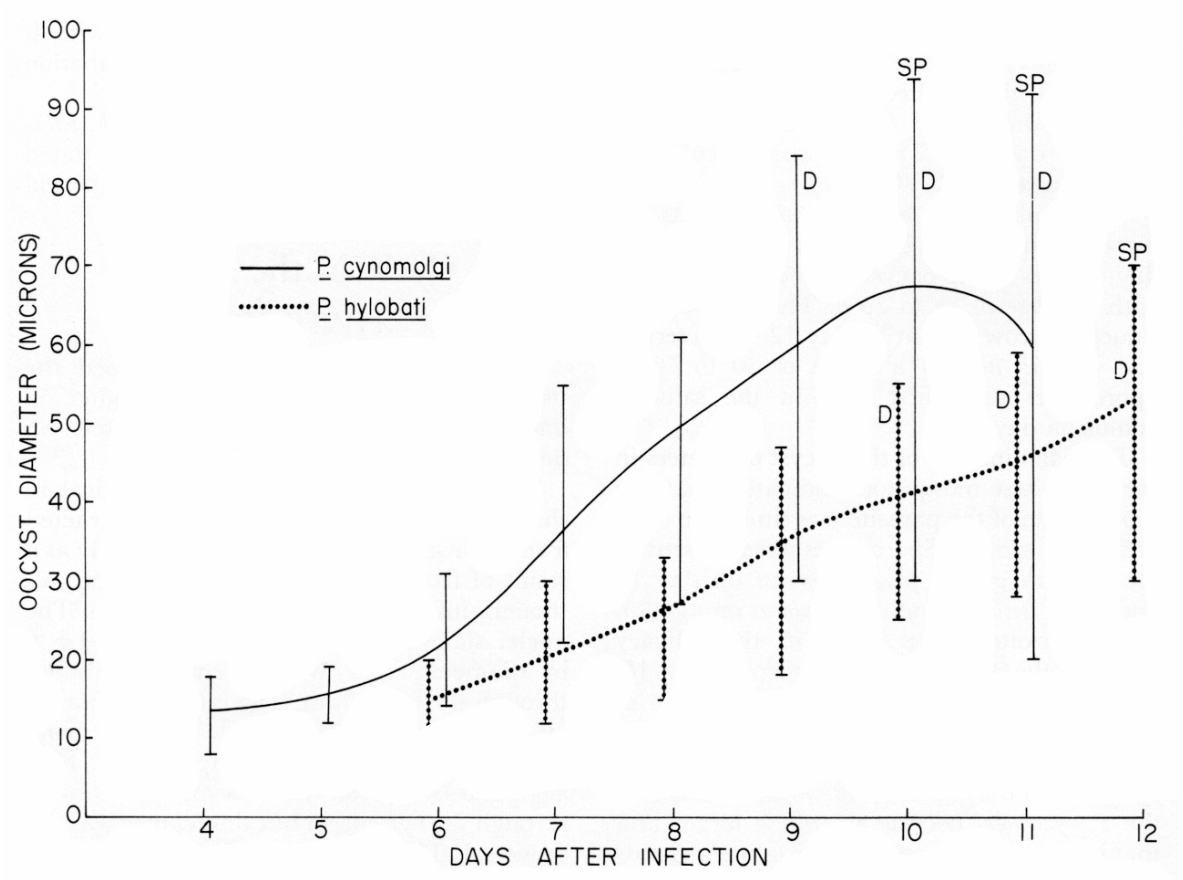


FIGURE 29.—Range in oocyst diameters and the mean oocyst diameter curve of *Plasmodium hylobati* and *P. cynomolgi* in *Anopheles b. balabacensis* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

acute inflammatory cell infiltrate was present.

The seven day forms (Figs. 1 & 2) sectioned at 3 µ extended through 5 sections; those sectioned at 6 µ, through 2.6 sections. The average size was 15.2 µ in length and 11.3 µ in width. The space occupied by the EE bodies averaged 15.8 µ in length and 11.7 µ in width. The limiting membrane was thin but distinct. Only two vacuoles were seen in fifty specimens examined; they measured 5 µ in diameter and were clear.

Only four EE bodies were seen in the 14-day material (Figs. 3 & 4). In the 6 µ sections they extended through 4 sections. Their average size was 26.1 µ in length and 18.6 µ in width. The space occupied by the parasites in the liver cell averaged 26.4 µ in length and 19.5 µ in width. A limiting membrane was distinct and flocculi were present, however, they were small and less frequent than in the 7-day forms. No vacuoles were present and vague clefts were observed in only one body.

Only two exoerythrocytic bodies were seen in the 7-day material from the owl monkey (Figs. 5 & 6). In the 6 µ sections one body was complete. It extended through 4 sections and measured 25.2 µ by 14.4 µ. Sections of the EE body measured up to 22.8 µ by 19.2 µ. There was a distinct limiting membrane. Flocculi were large and more frequent than in the gibbon material. The parasites exhibited small, clear vacuoles. The nuclei were round and measured 1-1.5 µ. Clefts were present in the cytoplasm.

It seems safe to say that the morphology of *P. hylobati* is not unlike that of the other primate malarias (Held *et al,* 1967). If one compares the 6-day parasite of *P. jefferyi,* the only other EE parasite of a gibbon so far described (Sodeman

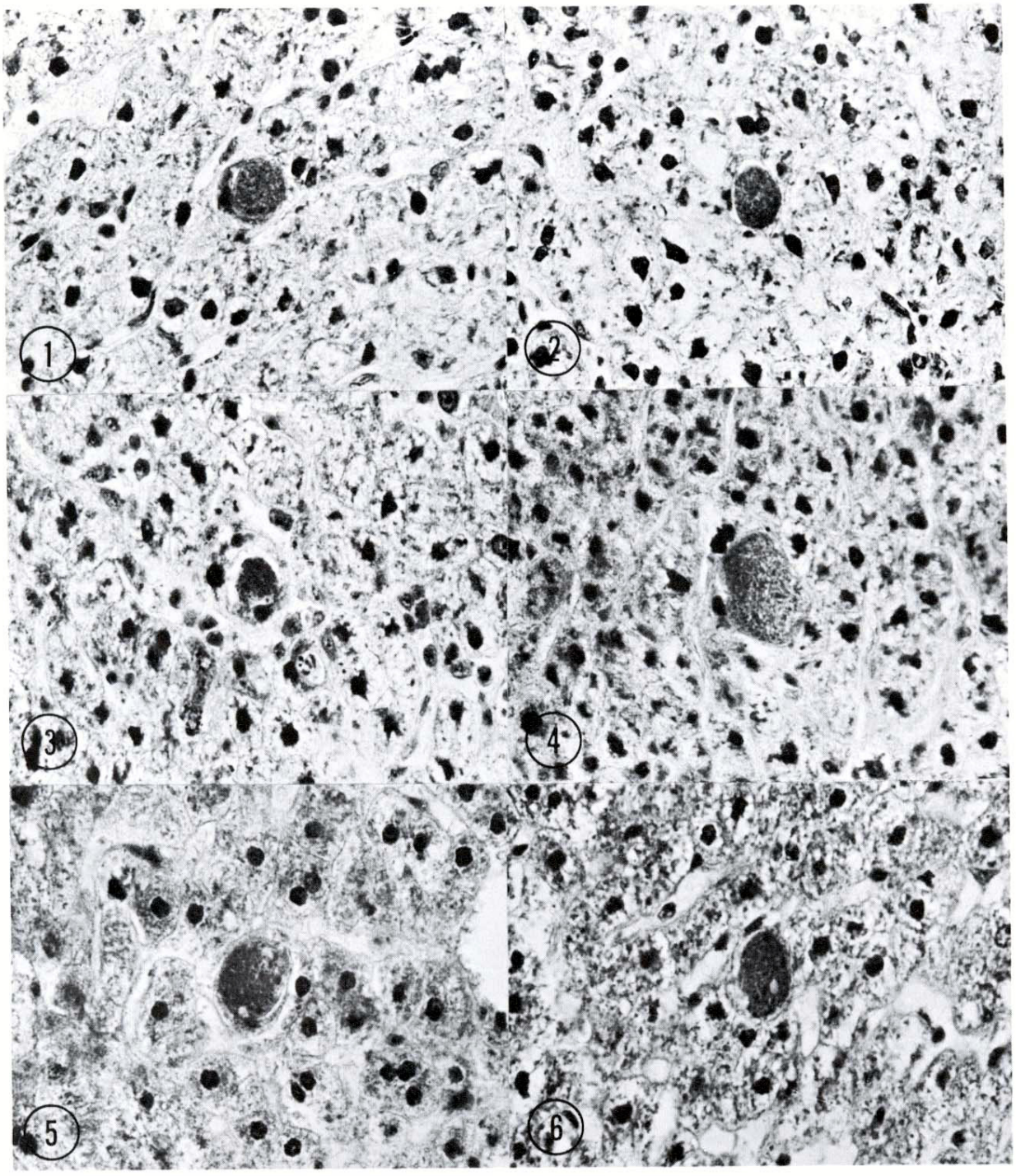


PLATE XIII.—Exoerythrocytic bodies of *Plasmodium hylobati*. X 580.

Figs. 1, 2. 7-day EE body in liver tissue of gibbon, *Hylobates moloch*.

Figs. 3, 4. 14-day EE bodies in gibbon.

Figs 5, 6. 7-day EE bodies in liver tissue of monkey, *Aotus trivirgatus.*

et al, 1969), with the 7-day forms of *P. hylobati* one sees considerable difference in the average size which suggests, if there were sufficient comparative measurements, that they might be separated at day 7 on the basis of size. The host animal exhibited a patent infection on day 9 yet EE bodies were found on day 14. We are not able to say, because of their large size, whether these were 'left-over' EE bodies of the initial generation, or, possibly, second generation EE bodies because some were of the same size as 7-day forms; maybe both. This will not be resolved until we have additional information on the tissue stages of this and other ape malarias.

Course of Infection

We know very little about the course of the infection in the normal host except that it does become latent and that it is provoked to exacerbation following splenectomy.

The parasite will infect splenectomized and intact rhesus monkeys. Infection induced by inoculation of parasitized blood in a splenectomized animal is marked by a very high parasitemia (up to 28/100 RBC) which persists at a detectable level for up to four months. Reinoculation has produced infections as high as 8/100 RBC. In intact rhesus, the infection is transient and is eliminated in a few weeks. Infections in *Macaca nemestrina* and *M. fascicularis* monkeys have also been obtained by the inoculation of parasitized blood. The parasitemias were of a low level. Gametocytes were produced, and numerous mosquito feedings were carried out; none of the mosquitoes became infected.

Host Specificity

The natural host of *P. hylobati* is the gibbon; infections have been reported in *H. moloch* from Java and North Borneo. Experimentally, *M. mulatta, M. fascicularis,* and *M. nemestrina* have been infected by the inoculation of parasitized blood.

Two human volunteers were exposed to infection through the bites of *A. b. balabacensis* mosquitoes heavily infected with this parasite. No patent infection was produced.

The natural invertebrate host of *P. hylobati* is unknown. On an experimental basis, five species of anopheline mosquitoes have been infected. *Anopheles b. balabacensis* was the most susceptible followed by *A. stephensi, A. freeborni, A. maculatus* and finally *A. quadrimaculatus.* The intensity of the infections varied from one mosquito species to another (Table 13).

TABLE 13.--Comparative infectivity of *Plasmodium hylobati* in *Anopheles b. balabacensis, A. stephensi, A. freeborni, A. maculatus, and A. quadrimaculatus.*

Mosq. species comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
Bal						100
Bal : St-1	2	20	15	100	100	49
Bal : F-1	2	20	20	100	90	43
Bal : Mac	2	20	29	100	100	22
Bal : Q-1	2	20	47	100	68	7

* Bal = *Anopheles b. balabacensis,* St-1 = *A. stephensi,* F-1 = *A. freeborni,* Mac = *A. maculatus,* 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. balabacensis* to another species where the GII of *A. balabacensis* = 100.

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