TABLE I.
Action of Tissue Fractions on Proplasmin.†

Tube No.	Reagents in cc.* Total extr.	Mitochondria	Supernate 1	Microsomes	Supernate 2	Diss. of clot (min.)
1	.1					15
2	.3					27
3		.1				30
4		.3				35
5			.1			16
6			.3			26
7				.1		11
8				.3		9
9					.1	120+
10					.3	120+

† All fractions were suspended in a volume equal to that of the original extract.
* Fibrinogen 0.2 cc + globulin solution 0.1 cc + 0.01M phosphate buffer 0.3 cc, thrombin (on glass rod) added to each tube. Temp. 37°C; pH 7.2.
Control experiments: 1. Same as in table with omission of globulin solution: no dissolution in 2 hr. 2. Same as in table with omission of fractions of tissue: no dissolution in 2 hr.

system described here may be identical with that proposed by Astrup and Permin.[7] It is known also that the microsome fraction of lung tissue contains most of the thromboplastic activity of the total tissue extract,[5] although this should not be construed as indicating an identity between the kinase and thromboplastin.

[7] Astrup, T., and Permin, P. M., *Nature*, 1947, **159**, 681.

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Exoerythrocytic Stages of *Plasmodium cynomolgi* in the *Macaca mulatta*.*

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The exoerythrocytic cycle from sporozoite to erythrocytic parasite has now been demonstrated in 4 species of avian malaria.[1-5] The exoerythrocytic stages, called cryptozoites and metacryptozoites by Huff and Coulston,[4] are widely distributed throughout the tissues of the avian host and develop in cells of the lymphoid-macrophage system and in endothelium.

Recently, Shortt and Garnham,[6,7] and Hawking[8] described pre-erythrocytic stages in the livers of monkeys (*Macaca mulatta*) in-

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[1] Huff, C. G., and Coulston, F., *Bi-monthly Report, Comm. Med. Res., Office of Scientific Research and Development*, 1943, No. 12, June 1.

[2] Reichenow, E., and Mudrow, L., *Deutsche tropenmed. Z.*, 1943, **47**, 289.

[3] Coulston, F., and Huff, C. G., *J. Infect. Dis.*, 1947, **80**, 209.

[4] Huff, C. G., and Coulston, F., *J. Infect. Dis.*, 1944, **75**, 231.

[5] Huff, C. G., Coulston, F., Laird, R. L., and Porter, F. J., *J. Infect. Dis.*, 1947, **81**, 7.

[6] Shortt, H. E., and Garnham, P. C. C., *Nature*, London, 1948, **161**, 126.

[7] Shortt, H. E., and Garnham, P. C. C., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1948, **41**, 785.

[8] Hawking, F., *Nature, London*, 1948, **161**, 175.

fected with sporozoites of *P. cynomolgi*. Later Shortt, Garnham, Covell, and Shute[9] found pre-erythrocytic forms of *P. vivax* in a liver biopsy of a man on the seventh day following infection with sporozoites. In the case of *P. cynomolgi* it was suggested that the exoerythrocytic stages developed in hepatic cells, and it was reported that these parasites were not observed in any other organ or tissue of the monkey. Although Hawking, Perry, and Thurston[10] described a pre-erythrocytic parasite in the brain of a monkey inoculated intracerebrally with sporozoites, they considered this parasite to be growing in an abnormal location. Further evidence was presented by Huff and Coulston[11] indicating that the pre-erythrocytic stages of mammalian malaria may develop in cells other than hepatic cells. They inoculated sporozoites of *P. vivax* into the liver of *M. mulatta* and observed cryptozoites in cells of the liver. These cells could not be definitely identified in their preparations but appeared to be either true endothelial cells or possibly cells of the lymphoid-macrophage system.

Materials and methods. Studies on the exoerythrocytic cycle of *P. cynomolgi* in *M. mulatta* have recently been initiated in this laboratory. The strain of *P. cynomolgi* used was received from Dr. R. J. Porter, School of Public Health, University of Michigan, in June 1946. Sporozoites were obtained from *Anopheles quadrimaculatus* which had been fed 15-19 days previously upon infected monkeys.

In these experiments a total of 12 monkeys have been used; 9 were infected with sporozoites, and 3 were controls. Pertinent data on these animals are presented in Table I. In some cases mosquito salivary glands, heavily infected with sporozoites, were inoculated directly into the liver.[4] Afterwards, this marked area was biopsied and serially sectioned. Sporozoites for intravenous inoculations were obtained by grinding in a glass mill either the thoraces of mosquitoes with intact salivary glands or the dissected salivary glands. A series of control experiments was done in which normal mosquito tissue was introduced into monkeys in the same manner as the infected material mentioned above.

The monkey tissues were fixed in Zenker-formalin, embedded in paraffin, and stained by either the Maximow method[4] or the modified Giemsa method described by Shortt and Cooper.[12]

Results. As a result of these investigations, pre-erythrocytic stages of *P. cynomolgi* have been found in the liver and spleen of *M. mulatta*. The experimental data are presented in Table I.

In *Exp. 1,* a monkey was inoculated intravenously with the ground thoraces of 178 heavily infected mosquitoes and was sacrificed and necropsied 6 days and 20 hours later. Upon histological examination of the tissues, several small segmenters and schizonts were observed in the liver in Kupffer cells (Fig. 1) and in the spleen in large mononuclear phagocytic cells, probably reticular in nature. No parasites were found in cells which could definitely be called hepatic cells, but this should not rule out the possibility that such cells may have been infected.

In *Exp. 2,* a monkey was inoculated twice directly into the liver with 18 and 24 heavily infected salivary glands. Biopsies of these inoculation sites, at 47 hours and at 89 hours, disclosed a few schizonts (12-18 μ) some of which appeared to be in Kupffer cells. These pre-erythrocytic stages were always found near the site of inoculation.

Nine days and 18 hours after inoculation, the day following detectable parasitemia, another liver biopsy was taken above the 47-hour inoculation site. In this specimen numerous parasites were found in the liver sinusoids in a circumscribed area. These stages were either schizonts and segmenters or merozoites free in the lumen of the sinusoid and were often associated with the Kupffer and littoral cells. Other parasites

9 Shortt, H. E., Garnham, P. C. C., Covell, G., and Shute, P. G., *Brit. Med. J.*, 1948, **1**, 547.

10 Hawking, F., Perry, W. L. M., and Thurston, J. P., *Lancet*, 1948, **ccliv**, 783.

11 Huff, C. G., and Coulston, F., *J. Parasitol.*, 1948, **34**, 264.

12 Shortt, H. E., and Cooper, W., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1948, **41**, 427.

TABLE I.

Biopsies and Necropsies of Normal Monkeys and Monkeys Infected with Sporozoites of *P. cynomolgi*.

Exper. No.*	Mode of sporozoite inoculation	No. of infected or uninfected mosquitoes used	Site of biopsy	Time of tissue collection: Between inoculation and biopsy — days	Between inoculation and biopsy — hr	Between inoculation and necropsy† — days	Between inoculation and necropsy† — hr	Pre-erythrocytic stages present	Remarks‡
				Infected Monkeys.					
1 A	Intravenous	178				6	20	Yes	Schizonts and few segmenters. Some in spleen.
2 A	Liver	24	Liver	1	23			Yes	Few schizonts. Parasitemia on 8th day.
B	”	18	”	3	17			Yes	Few schizonts.
C			”	9	18			Yes	Segmenters; biopsy taken near 2 A.
D			”	9	18			No	Biopsy of normal lobe.
E			Spleen	11	18			?	Large bodies resembling segmenters.
3 A	”	28	Liver	3	22			Yes	Schizonts and a few segmenters. Parasitemia on 9th day.
B	”	27	”	6				No	Injection site not located.
C						14		—	Unstudied.
4 A			”	1	20			Yes	Small trophozoites.
B	Intrav.	223				5	19	Yes	Schizonts in liver smears.
				Uninfected Monkeys.					
5 A			”					No	Biopsy of normal monkey.
B		0	Spleen					No	
6 A	Liver	20	Liver	2	3			No	Control to Exp. 2 and 3.
B	”	29	”	4	1			No	Control to Exp. 2 and 3.
C			”	10	1			No	Biopsy near 6 A.
D			Spleen	11	2			No	Tip of spleen.
7 A	Intrav.	180				5	20	No	Control to Exp. 1 and 4.

* Each experiment represents one monkey.
† All monkeys were sacrificed and tissue collected.
‡ All parasites are in liver unless otherwise noted.

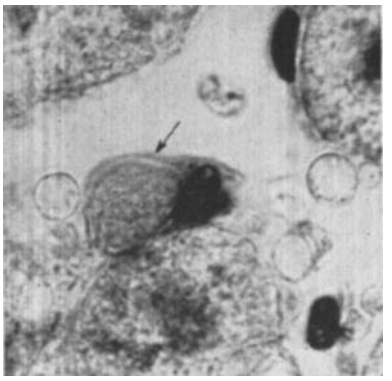

FIG. 1.
A pre-erythrocytic parasite of *P. cynomolgi* in a Kupffer cell, 6 days and 20 hours following the intravenous inoculation of sporozoites. Liver fixed in Zenker-formalin and stained by Maximow's hematoxylin, eosin-azur method. ×1300.

were found in cells which could not be identified; these may have been hepatic cells.

A splenic biopsy was also performed on this animal, 11 days and 18 hours after inoculation. Large bodies resembling schizonts and segmenters were observed in the venous sinuses, the pulp veins, and the trabecular veins. Despite this resemblance, the similarity of these forms to white thrombi raises a serious question as to whether they were exoerythrocytic stages. Heterophils, eosinophils, small and large lymphocytes, and monocytes were often observed surrounding and lying within these forms, resembling the leucocytic reaction which earlier investigators[7,10] had associated with segmenting exoerythrocytic stages of *P. cynomolgi*.

In *Exp. 3*, the liver of a monkey was inoculated in 2 places—in one site with 28 and in the other site with 27 heavily infected salivary glands. Liver biopsies were performed at 94 hours and at 144 hours but the inoculation site was located microscopically only in the 94-hour specimen. Schizonts and an occasional pre-segmenter were found in the 94-hour biopsy and ranged in length from 15-38 μ. These pre-erythrocytic parasites were primarily in Kupffer cells; some were definitely in hepatic cells. Three parasites were observed in the inoculation area in cells that could only be classified as large mononuclear phagocytic cells, similar to macrophages and Kupffer cells. The reaction area at the inoculation site was composed chiefly of degenerating liver cells, mosquito debris, heterophils, eosinophils, and a large variety of mononuclear phagocytic cells typical of an active inflammatory host response.

In *Exp. 4*, a monkey was inoculated intravenously with the ground thoraces of 223 heavily infected mosquitoes and was sacrificed 5 days and 19 hours later. Liver and spleen biopsies were made at 44 and 87 hours after inoculation. Air dried, Giemsa stained smears of the liver made at necropsy revealed the presence of 5 exoerythrocytic parasites, the largest of which had 26 chromatin bodies, the smallest 5. As is usual with such preparations, it was not possible to determine the type of host cell in which these parasites were located.

The parasites in these air dried liver smears contained vacuoles similar to those described for *P. cynomolgi*[7] and *P. relictum matutinum*.[13] However, no large vacuoles were seen in the histological preparations fixed in Zenker-formalin in any of the above experiments; clefts were observed.

In the biopsy made at 44 hours, small parasites resembling the small metacryptozoites of avian malaria were found in the Kupffer cells. This observation together with the findings of Experiment 2 suggest that cryptozoic segmentation occurred at about the 40th to the 48th hour.

A series of *control experiments* was performed to determine whether the observations mentioned above could be at all related to the parenteral inoculation of normal mosquito tissue. In this work, monkeys were inoculated, biopsied, and necropsied like the animals which received infected material. Tissues obtained from these animals were treated in the same manner as those from the infected monkeys. *Experiment 5* served as a normal control for the biopsies of infected liver and spleen. In *Exp. 6*, normal salivary glands were inoculated directly into the liver and then biopsied; this controlled Experiments 2 and 3. The monkey in *Exp. 7* controlled the infections, produced intravenously, in the

[13] Manwell, R. D., *Am. J. Trop. Med.*, 1940, **20**, 859.

animals of Exp. 1 and 4.

In none of these controls were bodies found which were similar to the pre-erythrocytic stages observed in the infected animals. The control material did demonstrate the need for caution in interpreting the data, for in these animals there were some forms such as degenerating liver cells and thrombi which might have been mistaken for parasites.

Discussion. The observations reported above suggest that the morphological characteristics of the pre-erythrocytic stages of *P. cynomolgi* are remarkably like those of the pre-erythrocytic stages of avian malaria. Certainly the tissue stages of *P. cynomolgi* are more similar to those of *P. gallinaceum* than of *Hepatocystes* (*Plasmodium*) *kochi*.[14] The exoerythrocytic stages of *Hepatocystes* resemble those stages observed in *Leucocytozoan* infections of ducks[15] while the *P. gallinaceum* and *P. cynomolgi* stages appear much like those of *Hemoproteus*. An obvious difference exists in that hepatic cells are not invaded by avian malaria and *Hemoproteus* whereas they may be in simian malaria, *Hepatocystes* and *Leucocytozoan*. Since *P. mexicanum* in the lizard[16] has both elongatum and gallinaceum type exoerythrocytic stages, it is to be expected that other species of malaria could have diverse host cell developmental potentialities.

Summary. Pre-erythrocytic stages of *P. cynomolgi* have been observed in Kupffer cells, hepatic cells, and mononuclear phagocytic cells of the liver, as well as in large mononuclear phagocytic cells of the spleen, probably reticular in nature.

[14] Garnham, P. C. C., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1948, **41**, 601.

[15] Huff, C. G., *J. Infect. Dis.*, 1942, **71**, 18.

[16] Thompson, P. E., and Huff, C. G., *J. Infect. Dis.*, 1944, **74**, 48.

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Determination of Carbon 14 in Fatty Acids by Direct Mount Technic.*

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The measurement of C^{14} in fatty acid fractions prepared from animals into which a C^{14}-labeled fatty acid has been introduced is beset with considerable difficulty. A sample of high specific activity is not often obtained because the administered radioactive fats are diluted by a factor of at least 1000 when mixed with the body pool of fatty acids. The activity per unit of mass is further reduced by a factor of 17 when the conventional $BaCO_3$ technics are used. The first dilution—that due to mixing with the body pool of fatty acids—obviously cannot be avoided. In order, however, to circumvent a further reduction of the activity, it seemed desirable to investigate direct mounting of fat samples.

When samples of C^{14}-containing fatty acids were mounted directly on bare aluminum discs, reproducible values for counting rates were not obtained because the material collected on the surface of the discs in the form of globules. Reproducible results were obtained by employing a cover of lens paper which brought about an even distribution of the fatty acids on the disc. The degree of reproducibility that can be attained by mounting radioactive fatty acids on lens paper-covered aluminum discs is shown in Table I which records the results of quadruplicate determinations for each of 3 different fatty acid samples. The deviations from the averages did not exceed 5%. The degree of reproducibility which can be at-

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