

SubTitle: Comparative Studies in the Pathology and Host Physiology of Malaria: Gibbon Malaria.

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Four plasmodia have been described as natural infections of S.E. Asian gibbons: Plasmodium hylobati (Rhodain, 1941), P. youngi (Eyles, et al 1964), P. eylesi (Warren et al, 1965) and P. jefferyi (Warren et al 1966). In addition, Hylobates lar may serve as an experimental host for P. falciparum (Ward et al, 1965; Ward and Cadigan, 1966). Virtually nothing is known of the host pathophysiology in these infections. Eyles et al. (1964) noted that gibbons infected with P. youngi showed a clinical illness fever, anaemia and lethargy.

The species of plasmodium employed in this present study cannot be given with certainty. We have observed forms corresponding to all four species occurring in any individual during the course of its infection. There may be some confusion in the taxonomy of gibbon plasmodia and an account of the morphology of the Thai isolate employed in this study will appear elsewhere. Despite this deficiency, it is felt that the host response of the gibbon is sufficiently unique to warrant this present paper.

METHODS

Adult Hylobates lar of Thai origin were used in these experiments. The principles of animal care as promulgated by the National Society for Medical Research were observed. Prior to the experiment, blood films were taken from each animal and only when negative for malaria parasites was the gibbon used. All animals were then given a full course of 4 amino and 8 amino-quinolines and experimentally infected not earlier than 3 months after completion of the chemotherapeutic regimen. Splenectomy was performed between one and ten months before experimental infection.

The plan of study and methods followed that for the P. coatneyi malaria (Desowitz et al, 1967). Five splenectomized (S2, S4, S14, S28, and P14) and one intact gibbons (P16) were infected by intravenous or intraperitoneal inoculation of parasitized blood. The inoculum ranged from 6.0×10^6 to 5.5×10^8 parasites. The plasmodium employed in this study was isolated from a naturally infected H. lar from Tak, Northwest Thailand. Seven ml. of blood was obtained weekly for haematology and serum chemistries (transaminases, alkaline phosphatase, direct and total bilirubin, blood urea nitrogen, and creatinine). Temperature and blood films for parasite density were taken daily. Bone marrow aspirations and biopsies of liver and kidney were made on selected animals.

Two control animals were maintained and haematology and blood chemistries obtained in the same manner as for infected animals.

RESULTS

Controls. Tables I and II show the weekly values for two uninfected gibbons over a period of 56 and 69 days. Table III summarizes the preinfection values for all experimental animals. While there was the expected variation of weekly haematologic values there was no indication of anaemia produced by the successive bleedings. Variations serum chemistries in individual animals over a period of time and between animals were of similar magnitude to those previously found for normal rhesus monkeys (Desowitz et al, 1967).

Infected gibbons.

The course of parasitaemia and host response were similar in all gibbons studied. Fig. 1 shows the parasitaemia, haematology and blood chemistries for one typical infection while fig. 2 presents the parasitaemia, haematocrit and serum cholesterol for all experimental animals. It will be seen from these figures that the outstanding features of this infection were a high unrelenting parasitaemia, severe persistent anaemia and hypocholesterolaemia.

There was a close relationship between parasitaemia and anaemia. This was further exemplified in S14, a gibbon given two courses of chloroquine treatment. At each reduction of parasitaemia there was a rapid increase in haemoglobin and haematocrit. The one exception to this pattern was P16, an intact gibbon. This animal recovered from the anaemia despite the continuing parasitaemia of a similar magnitude as that in splenectomized gibbons,

The continuously high indirect bilirubinaemia (0.5-1.8 mg per cent) reflected the haemolytic process. There was no indication of severe bone marrow depression as an added factor in the etiology of the anaemia. The marrow showed erythroid hyperplasia with an M:E ratio of 1:2 rather than the normal ratio of 2:1. There was a reticulocytosis that occasionally attained 40 per cent and numerous nucleated red blood cells appeared in the peripheral blood. There was no depression of the white blood cell count.

Despite the intense, persistent anaemia most animals showed no overt signs of illness. They continued to eat and were not lethargic. S4, the only fatal infection encountered, appeared well until it sustained a parasitaemic recrudescence with rose from approximately 100 parasites per 50 thin film fields on the 153rd day to 600 parasites on the 158th day. This caused a further drop in the haematocrit, from 14 to 5 per cent, and the animal died.

The marked hypocholesterolaemia appeared to be associated with the anaemia. As will be noted from fig. 2 the rapid fall in cholesterol closely paralleled the progression of anaemia and returned toward normal levels only when the anaemia improved, such as in P16 and S14.

Little other serum chemistry abnormality was evident at any stage of the infection. There was no indication of hepatic abnormality. The transaminases showed no elevation and were remarkably constant during the entire period of observation in all the animals as exemplified in fig. 1. Furthermore, the two tests for liver function, direct bilirubin and alkaline phosphatase, were not elevated. Liver biopsies obtained on S4 (85th day of infection) and P14 (135th day) showed no features suggestive of functional alteration. The Kupffer cells contained large masses of malarial pigment. The hepatic cell cytoplasm in S4 was irregular with clumping and vacuolization, more prominent near the central vein. Renal abnormalities were also absent as indicated by normal BUN and creatinine values. There was, in fact, a tendency for BUN to be lowered as the anaemia progressed. Renal biopsy on P14 showed no histopathology indicative of significant functional alterations. There was moderate swelling of the glomerular endothelium.

As in P. coatneyi infections, there was a trend toward lowered alkaline phosphatase levels during the infections. In some gibbons, such as S2 in which the alkaline phosphatase fell from 23 to 7 units, this was striking.

Discussion

Comparison of the pathophysiology of P. coatneyi in the rhesus monkey and gibbon malarial brings into focus two distinct pathologic conditions; acute P. coatneyi malaria, characterized by anaemia plus a "toxic" element which manifests itself in hepato-renal pathology and gibbon malaria in which there was an intense anaemia without any indication of other pathologic alterations. The parasitaemias in the splenectomized gibbons were at least as high as those in the acutely ill monkeys with P. coatneyi.

Furthermore, these high parasitaemias persisted for much longer periods than in the P. coatneyi infected rhesus. The ability of the gibbon to sustain the burden of profound anaemia for long periods of time without overt signs of illness or secondary tissue pathology is truly remarkable. The underlying mechanism responsible for the development of organ lesions in malaria is not known with certainty. Maegraith (1948) has proposed that the combined effects of anoxaemia due to anaemia and haemodynamic circulatory changes induce the tissue pathology. With regards to the genesis of circulatory disturbances and consequent histotoxic effect he states, "I think changes in the permeability of the vascular endothelium are of supreme importance". Certainly anaemia per se, as indicated by this present study, did not produce observable organ pathology. It may be relevant that we have not been able to demonstrate in gibbon malaria the serum vascular permeability factor present in P. inui and P. coatneyi infections (Desowitz and Pavanand, 1967). However, the study is still in its early stages and this observation requires further confirmation. Fundamentally, we are again confronted with the old problem of virulence in host-parasite relationships. Is P. coatneyi more virulent than gibbon malaria, i.e., are there inherent differences in the pathogenic potential between parasite species; or is the difference attributable to a peculiarity of host response in gibbon and rhesus? Undoubtedly both factors contribute to the complex production of disease. Virulence might be defined as an expression of interacting parasite and host physiologies. Virtually nothing in known of the metabolism of the metabolism of gibbon or other primate plasmodia, and the meagre data available cannot, as yet, be rationally applied to an understanding of disease production.

The most striking chemical change induced by gibbon malaria was a rapid and persistent fall in serum cholesterol. Hypcholesterolaemia has been observed in human malaria (Crespin and Zaky, 1919; Fairley and Bromfield; 1933 McQuarrie and Stoesser, 1932; Kehar, Kopp and Solomon, 1943) in chronic P. knowlesi infections (Krishnan. et al, 1936: Kehar, 1937), and in P. coatneyi malaria (Desowitz et al, 1967). The reduction of serum cholesterol is probably common to many infectious diseases. McQuarrie and Stoesser (1932) noted a definite fall in cholesterol during pneumonia, empyema, tonsillitis and otitis media.

At present we can only speculate as to the cause of hypcholesterolaemia but several possible explanations can be offered. These are; (1) dietary deficiency, (2) depletion from utilization by parasites and/or reticulocytes and reticuloendothelial elements, (3) impaired synthesis and (4) enhanced catabolism. The precipitous fall in the cholesterol level would tend to obviate a dietary etiology since a reduced food intake does not appreciably alter serum cholesterol (Keys et al., 1950).

Depletion of cholesterol from incorporation into parasites or reticulocytes is a possibility although it would seem to be more of a contributory factor than a primary one. Morrison and Jeskey (1947) have shown that almost 30 per cent of the dry weight of P. knowlesi is material, predominantly cholesterol. More recently however, Wallace et al. (1965) found sterols in P. lophurae and P. berghel to be in a lower proportion than the phospholipids. Because of the similarity in cholesterol concentrations in P. knowlesi and host blood Williamson and Ginger (1965) postulated a direct uptake of sterols by the parasite from host blood. Reticulocytes may also play a role since these cells contain relatively large amounts of cholesterol, two to three times that of mature erythrocytes (Raderecht et al., 1960). It has been clearly demonstrated that in gibbon malaria the hypcholesterolaemia is coincidental with the anaemia and reticulocytosis, but the nature of this association has not been elucidated. A search of the literature for other investigations relating to cholesterol level in haemolytic anaemias has not been productive. The increased activity and hyperplasia of reticuloendothelial elements during malaria is well known. Riggi and Di Luzio (1962) have shown that the stimulated reticuloendothelial system caused a reduction in hepatic and plasma ester and total cholesterol. Thus, this factor also could contribute to the hypcholesterolaemia of malaria.

We have no direct information as yet pertinent to the third possibility, decreased synthesis. Impairment of cholesterol synthesis is known to occur in diseases affecting the liver parenchyma but there was no biopsy or serum chemistry evidence of this in the infected gibbon. Moreover, in the acutely infected P. coatneyi rhesus with centrilobular necrosis the decrease in serum cholesterol was less in the than gibbon malaria. In this present investigation only total cholesterol was analyzed. Determination of esterified and free

cholesterol ratios should help clarify the question whether the liver is responsible for the hypocholesterolaemia. The fourth hypothesis, increased cholesterol catabolism, also remains to be investigated.

The hypocholesterolaemia itself may contribute to the etiology of the anaemia. Murphy (1962) has shown that cholesterol-depleted erythrocytes have an increased osmotic fragility. The cholesterol in the mature erythrocyte is in dynamic equilibrium with the serum nonesterified cholesterol (London and Schwarz, 1953) and presumably a state of hypocholesterolaemia would lead to a depleted erythrocyte.

Summary

- 1) Five splenectomized and one intact H. lar were inoculated with a plasmodium isolated from a naturally infected gibbon from Northwest Thailand.
- 2) The pathophysiology of the infections was studied in terms of pararitaemia, haematology blood chemistry, and histology.
- 3) The outstanding features of this infection were a high, unrelenting parasitaemia, severe persistent anaemia and hypocholesterolaemia. The hypocholesterolaemia coincided with the anaemic state.

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TABLE I — Haematology and blood chemistries of serial samples from control gibbon S73

Days	Haematology						Blood Chemistry							
	WBC /mm ³	RBC x10 ⁶ / mm ³	Haemo- globin gm%	Haemato- crit %	Reticu- locytes %	Bilirubin mg%	SGOT S.F. Units	SGPT S.F. Units	Alkaline Phospha- tase Sigma Units	BUN mg%	Creati- nine mg%	Choles- terol mg%	Bilirubin mg%	
													Direct	Total
0	9,600	5.62	11.2	37	0.6									
6	5,400	5.88	12.1	38	0.4	0	35	21	22.0	11.2	0.9	113	0	0
13	6,400	5.36	9.9	33	0.8	0	25	23	29.2	11.7	0.8	112	0	0
20	4,700	6.52	10.3	35	0.9	0.05	22	18	22.0	14.2	1.0	111	0.05	0.2
27	6,100	5.40	9.5	31	0.4	0	31	16	22.0	5.9	0.7		0	0.4
35	6,900	5.45	10.8	33	0.3	0	19	18	23.6	13.7	0.9	89	0	0
41	5,400	6.54	11.0	37	0.1	0.1	20	19	28.8	10.4	0.9	112	0.1	0.2
48	5,500	6.30	10.4	36	1.1	.05	26	25	26.8	18.3	1.0	113	.05	0.3
63	5,000	5.90	12.2	41	1.1	0	22	17	30.0	12.1	0.8	129	0	0.1
69	6,200	6.40	13.8	36	0.2	0	22	17		13.8			0	0
Range	4,700— 9,600	5.36— 6.54	9.5— 13.8	31—41	0.1— 1.1	0— 0.1	19— 35	16— 25	22.0— 30.0	5.9— 18.3	0.7— 1.0	89—129	0— 0.1	0— 0.4

TABLE II — Haematology and blood chemistries of serial samples from control gibbon S62

Days	Haematology						Blood Chemistry						
	WBC /mm ³	RBC x10 ⁶ /mm ³	Haemo- globin gm%	Haemat- ocrit %	Retic- ulocytes	Bilirubin mg%	SGOT S.F. Unite	SGPT S.F. Unite	Alkaline Phospha- tase Sigma Units	BUN mg%	Creati- nine mg%	Choles- terol mg%	
													Direct
0	6,100	6.21	11.0	38	0.4								
7	4,600	6.09	10.5	38	0.2	0.1	43	44	26.6	13.7	1.5	186	
14	6,100	5.87	10.6	39	0.5	0.3	46	35	19.0	11.4	1.1	170	
21	7,800	6.90	11.3	41	0.4	0	52	41	14.6	11.5	0.7	188	
28	6,300	6.37	11.0	38	0.2	0	48	32	25.0	10.0	0.9	170	
35	9,900	6.20	11.0	38	0.7	0.05			16.0	12.3	0.8	142	
42	6,600	5.79	10.8	36	0.4	0	40	36	16.0	14.9	0.8	154	
49	8,000	6.23	11.2	37	0.4	0.05	39	26	19.8	11.2	0.8	138	
56	5,600	6.32	10.1	39	0.8	0.1			14.8	12.9	1.1	150	
Range	4,600— 9,900	5.79— 6.90	10.1— 11.8	36—41	0.2— 0.8	0— 0.4	39— 52	26—44	14.6— 26.6	10.0— 14.9	0.7— 1.5	138—188	

TABLE III — Summary of preinfection values of experimental gibbons

Gibbon	Haematology						Blood Chemistry						
	WBC /mm ³	RBC x10 ⁶ / mm ³	Haemo- globin gm%	Haemato- crit %	Retic- uloocytes	Bilirubin mg%	SGOT S.F. Units	SGPT S.F. Units	Alkaline Phospha- tase Sigma Units	BUN mg%	Creati- nine mg%	Choles- terol mg%	
													Direct
	8,800	6.12	13.4	45	0.4	0.05	30	26	23.2	6.6	1.1	154	
	13,100	5.73	12.7	44	0.4	0	34	31	19.0	9.8	1.1	155	
S2	11,700	7.14	13.1	44	0.8	0.05	21	20	23.6	10.2	1.0	160	
S9	7,800	4.49	11.8	39	0.9	0.05	27	26	19.0	2.7	0.6	111	
S4	6,500	5.71	11.4	38		0.1	38	37	15.0	19.7	0.7	130	
S65	4,600	6.50	10.1	36	0.6	0	34	19	18.2	15.2	0.6	121	
P14	7,200	5.73	12.7	41		0.05	36	28	18.0	17.0	1.2	148	
S28	7,900	7.07	13.6	45			39	23	38.0	13.0		145	
P16	5,900	6.60	13.4	43			43	28	14.0	11.0		174	
S14	16,900	5.65	12.2	42	0.5	0.05	32	29	31.6	11.7	1.0	128	
	16,100	5.82	12.1	41	0.9	0.1	31	26	27.2	14.2	0.9	130	
	12,500	6.91	12.7	43	0.7	0.05	29	25	31.2	12.9	0.9	131	
Range	4,600 16,900	4.49- 7.14	10.1- 13.6	36-45	0.4- 0.9	0- 0.1	21-43	19-37	14.0- 38.0	2.7- 19.7	0.6- 1.2	111-174	

Fig. 1. Parasitaemia, haematology and blood chemistries during a typical course of infection in a splenectomized gibbon.

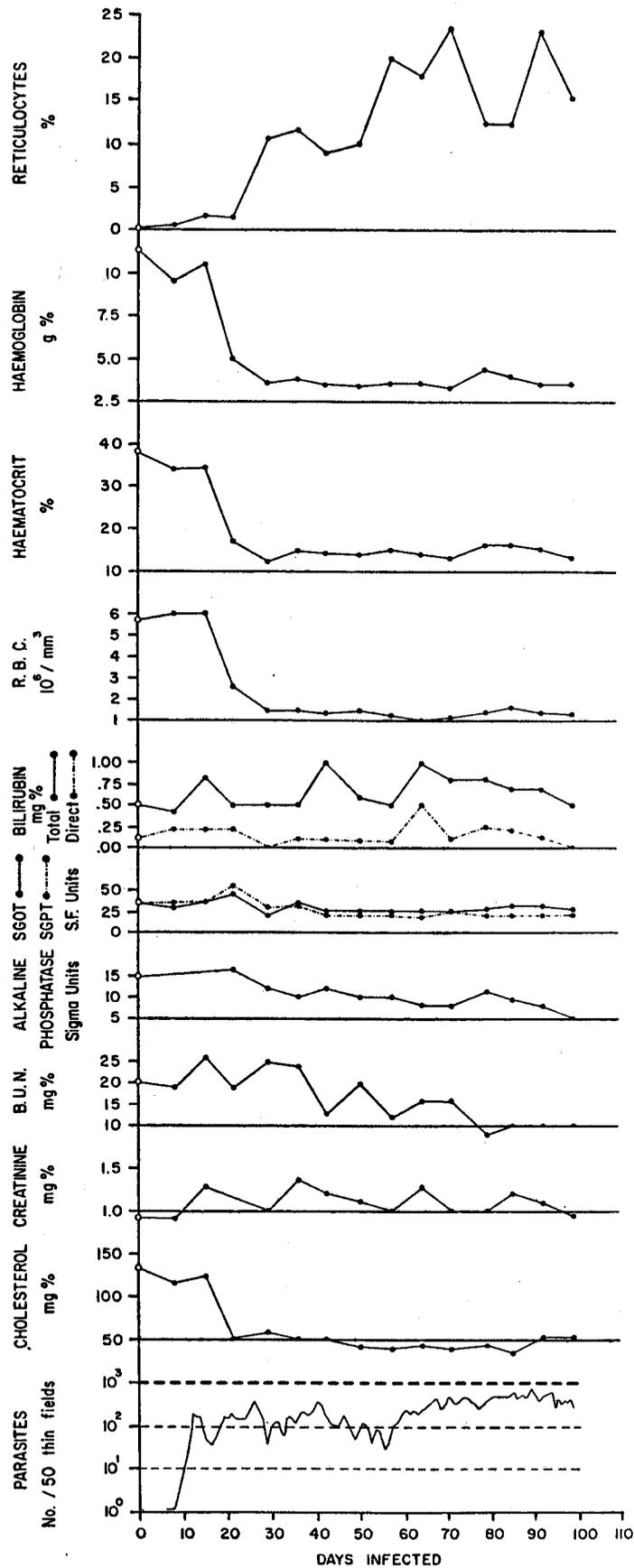


Fig. 2. Parasitaemias, haematocrits and cholesterol levels during the course of infections in all experimental gibbons.

