Relationship of Serum Complement Levels to Events of the Malarial Paroxysm

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ABSTRACT Malarial paroxysms due to *Plasmodium vivax* were studied for alterations in whole serum complement (C′) and certain C′ components. The objective was to relate C′ values with events of the parasite cycle during schizogony and with the febrile pattern. Substantial decreases in C′ were found in 9 of 18 paroxysms studied during relapse. In contrast, only one of 22 paroxysms occurring during the primary attack was associated with a striking depression in C′, and this case exhibited certain characteristics of a relapse paroxysm. The mean change in C′ levels during paroxysms in relapse (−23%) was significantly different from paroxysms of the primary attack (−2%). Depletion of C′ was associated directly with degree of parasitemia and presence of complement-fixing (CF) antibody. Lowest levels of C′ were found within a few hours after completion of schizont rupture and peak fever. C4 levels reflected changes in whole serum C′ and appeared to be a more sensitive indicator of C′ alterations during malaria. While the alterations in C4 as well as C1 and C2 indicated that the classical C′ pathway was involved, some preliminary results showed little or no depletion of late components, C3 and C6. Overall results are compatible with C′ activation and depletion during or soon after schizont rupture if parasite density is sufficiently high and if CF antibody is present.

INTRODUCTION

The responsible factors are still unknown in pathogenesis of the malarial paroxysm, with its dramatic clinical features including sudden rigor, severe headache and myalgia, and rapidly rising fever. Although a general relationship between rupture of parasitized cells and ensuing fever in malaria has been recognized for years (1), the actual mechanism by which schizont rupture triggers these clinical events is not understood. In fact, even precise definition of the time interval between the terminal phase of schizogony and initial clinical manifestations of the paroxysm are not available from the literature. Cognizance of the need for a better explanation for many basic clinical elements of human malaria encouraged us to investigate pathogenesis of the malarial paroxysm.

One aspect of our investigations was the time-course of schizogony in relation to clinical events; these findings will be reported separately.[1] Since certain types of immunologically mediated pathology in malaria have been suggested (2), and in view of the fact that the complement system has been implicated increasingly in various pathological processes (3), we decided to study serum complement (C′)[2] over the time-course of the malarial paroxysm. The induction of experimental infections for other purposes in volunteers at the NIH Malaria Project at the Federal Penitentiary in Atlanta, Ga. made most of these studies possible. Some patients with naturally occurring malaria at the NIH Clinical Center were also studied. In all cases, however, observations on the degree of parasite synchrony and febrile patterns permitted selection of well-synchronized infections for study.

Although previous literature on C′ during malaria will be discussed later, the subject has received considerable attention and several reports are especially pertinent. As long ago as 1922, Radosavljevic concluded from

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[1] Neva, F. A., et al. Unpublished studies.

[2] *Abbreviations used in this paper:* C′, whole serum complement; CF, complement fixation (fixing): $C'H_{50}$, 50% hemolytic units.

semiquantitative tests then available that serum C′ activity was reduced during the paroxysm in human malaria (4). In 1948 with the use of quantitative assays for C′, Dulaney reported a general association of reduced C′ in patients with malaria (5). More recent studies in heavily infected monkeys have found a striking depletion of C′ shortly before death (6), and variation in C′ and C′ components levels during schizogony (7). However, no previous reports have related alterations of the C′ system in man to the time-course of fever and parasite development during the malarial paroxysm and to malarial immunity.

In this study evidence is presented for transitory depletion of C′ during paroxysms of vivax malaria when parasitemia is high and antibody is present. The conditions and time-course in which decreases in C′ could be demonstrated are compatible with antigen release and complement consumption via the classical pathway during or very soon after schizont rupture. Yet, there appeared to be little or no depletion of some late-reacting components of complement. In two other papers (8, 9) these findings were confirmed and studied in greater detail with regard to complement components involved in rhesus monkeys infected with *Plasmodium coatneyi.*

METHODS

Malaria infections. Most subjects were inmate volunteers in the NIH malaria research program at the U. S. Federal Penitentiary, Atlanta, Ga. with malaria infections induced for purposes of other studies. Before participation the nature of the experiment, hazards, and procedures required were carefully explained to the inmates and no coercion or excessive inducements were offered for their voluntary participation in the studies. All volunteers were adult males less than 40 yr of age and were screened for mental and physical fitness with a medical history, physical examination, chest X ray, electrocardiogram, and laboratory tests that included glucose-6-phosphate dehydrogenase level and assessment of liver and renal function. Three of the subjects, also adult males ages 20–35 yr, with naturally occurring malaria were studied in the NIH Clinical Center.

Infections were induced by the bites of infected mosquitoes (*Anopheles freeborni, A. balabacensis. A. maculatus,* or *A. quadrimaculatus*). The species of malaria for most volunteer and all natural infections was *Plasmodium vivax;* the strains included Chesson from New Guinea, Central American strains from Nicaragua, Salvador, and Panama, and one from South Vietnam. The three natural infections were acquired in South Vietnam and the New Guinea area.

When routine blood smears disclosed patent parasitemia, volunteers were admitted to the malaria ward where oral temperatures were taken at 4-h intervals. When fever of 100°F or greater was found, temperatures were taken hourly. In most of the patients temperatures were recorded with a rectal probe thermistor thermometer at 15–60 min intervals before and during a paroxysm designated for study of complement levels.

Giemsa-stained thick and thin smears of peripheral blood, taken at least daily and at intervals as frequently as every 30 min during particular paroxysms, were used to determine parasite synchrony and density. The latter determination was obtained from thick smears by multiplying the number of parasites per 100 leukocytes by a factor derived from the daily leukocyte count (leukocytes per $mm^3 \div 100$); parasite densities were expressed as parasites per cubic millimeters of blood.

The time of peak fever was used as the main objective indicator of the paroxysm to which other events could be related; blood smears and serum samples were collected frequently over the course of febrile paroxysms. Since it was impossible to predict when peak fever would be reached, the times at which blood specimens were taken were not identical in each patient with relation to time intervals before or after peak fever. Therefore, to calculate differences in C′ levels which occurred during a paroxysm, it was arbitrarily decided to compute changes from specimens drawn within a period of 10 h before and after peak fever. Further, in interests of uniformity and objectivity, the change in C′ level for each paroxysm was computed from the lowest value available up to ±0.5 h of peak fever as compared to the lowest value up to 10 h after peak fever.

Each episode of patent parasitemia was terminated by appropriate antimalarial therapy. Only rarely were blood samples for complement determinations taken after initiation of chemotherapy, but our results gave no indication that complement levels were influenced by administration of chloroquine or quinine.

Serologic procedures. Blood for serum complement determinations was allowed to clot in sterile tubes at room temperature for at least 30 but no longer than 90 min. It was then either stored at 4°C for not more than 1 h before separation of serum or centrifuged directly at 4°C for 15 min and serum was transferred in multiple portions of 0.5–3.0 ml to sterile screw-cap glass vials and stored at −70°C. The period of time between drawing of blood samples and storage of serum at −70°C did not exceed 3 h. When transportation or sorting of serum samples was necessary they were packed in liberal quantities of dry ice and maintained in the frozen state. Specimens for determination of whole serum complement (C′) were thawed only once at the time the assay was performed, but some samples tested for C4, C1, and C2 had been carefully thawed, subdivided in multiple specimens, and promptly refrozen and stored at −70°C before final thawing and assay.

The assay procedure for C′ was basically that described by Hook and Muschel (10) and hemolytic activity was expressed in terms of lysis of 50% of 2.5×10^8 optimally sensitized sheep red cells. The diluent was borate-buffered saline containing Mg^{++} and Ca^{++}, the final reaction volume was 1.5 ml, and the percent hemolysis was determined spectrophotometrically. A probit plot (volume of diluted test serum on a log scale vs. percent hemolysis) was constructed from two and preferably three values from tubes yielding between 15 and 85% hemolysis. The C′ titer was determined from the intercept of the above plot at the 50% level. The titer of C′ represented the number of 50% hemolytic units ($C' H_{50}$) in 1 ml of undiluted text serum. All serum specimens for a given paroxysm or series of paroxysms over a few days were tested simultaneously.

Activity of C4 was determined by a hemolytic assay based upon the ability of the test serum to reconstitute complement activity to serum from guinea pigs with total genetic deficiency of fourth component of complement. The procedure has been described in greater detail elsewhere (11), but it essentially involves addition of a sufficiently high dilution of test serum to furnish only one effective C4 site per red cell to an excess of C4-deficient guinea pig serum, stopping the reaction after 2 h with EDTA, and recording

lysis spectrophotometrically. C3 and C3 proactivator were analyzed immunochemically by radial diffusion with Hyland plates (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) and M-Partigen C3 activator plates (Behringwerke A6, Marburg-Lahn, West Germany) containing the appropriate reagents. The methods for estimating C6, C1, and C2 were all hemolytic assays, the former utilizing C6-deficient rabbit serum and the latter by standard procedures which are described elsewhere (9). With each assay procedure all test specimens for a given paroxysm were tested simultaneously.

In complement-fixation (CF) tests for CF antibody, a microtiter modification of the procedure described by Kent and Fife (12) using 6.0 U of complement and a 50% hemolytic end point was employed. Volume of reactants varying from 0.025 to 0.05 ml were added for overnight fixation, a 2% red cell suspension spectrophotometrically standardized was added, and the results were read and compared visually to appropriate standards. Sera were tested at twofold dilutions in a volume of 0.025 ml and beginning at a 1:2 dilution, with final CF titers representing the reciprocal of the dilution of serum that gave 50% hemolysis. The antigen used in the CF test was an aqueous soluble fraction from heavily parasitized red cells of a rhesus monkey infected with *P. cynomolgi*. CF antigen was prepared by repeated washings of parasitized red cells by centrifugation, lysis with saponin, and separation from red cell ghosts and membranes by centrifugation, treatment in a Hughes press, and finally passage through a 0.2-μm filter and lyophilization. The antigen was stored in the lyophilized state at −70°C until used and optimum concentration for its use in the CF test was first determined by block-titration with a known reactive serum from a patient infected with *P. vivax*. At least two serum samples from each patient, one just before and the other at the end of a paroxysm during the primary attack or during relapse, were tested simultaneously for CF antibodies.

RESULTS

Clinical course of malaria in patients studied. It must be remembered that the initial febrile pattern exhibited by the malaria patient during the primary attack is usually irregular. It may be sustained or consist of daily fever spikes. Within about a week the fever pattern tends to revert to its classical pattern of sharp elevations every other day with alternate days without fever in the case of *P. vivax* and *P. falciparum* infections. In contrast, during the later relapses which may occur with *P. vivax* infections, the fever pattern from the start is more likely to show the alternate day cycle. The chill and subsequent fever reflect the degree of synchrony in the developmental cycle of the malaria parasite. When all parasites are "in phase" and undergo their development at the same time, the infection is said to be well-synchonized. Daily fever spikes in *P. vivax* infections are considered to represent two asynchronous populations or broods of parasites and sustained fever probably indicates the existence of more than two broods. Therefore, infections referred to as *primary attacks* in this report represented the first clinical evidences of malaria within 2–3 wk after exposure to infected mosquitoes. The febrile paroxysms studied were discrete daily temperature elevations but were not completely synchronous in that paroxysms did not regularly occur at 48-h intervals. The term *relapse* refers to clinical activity occurring about 16 days or longer after treatment of a primary attack with chloroquine or quinine, and paroxysms during relapse were generally well synchronized at 48-h intervals.

Base-line complement values. Whole C′ values were available from 16 volunteers from specimens collected on the 9th day after exposure to infected mosquitoes but before parasites, fever, or symptoms had appeared. The mean titer, expressed as number of calculated 50% hemolytic units per ml of serum, was 191 with a range of 89–294 and an SEM of 14.

Whole serum complement during relapses. A total of 18 paroxysms in 12 patients with relapses of *P. vivax* were studied in which at least three C′ levels were available before and after peak fever spanning a period no greater than 14 h. Four to six blood specimens for C′ during a paroxysm were obtained in many cases. The change in whole serum C′ during these 18 paroxysms varied from +8 to −72% with a mean drop in C′ level of 23%. In some instances the decrease in C′ levels over a period of a few hours was striking (e.g. −72, −63, and −62%) and in 9 of the 18 paroxysms was >20%. More detailed results of the change in C′ levels with time during paroxysms in relapse are presented in Table I for representative cases. In addition, it was noted that values for C′ early in the paroxysms before peak fever tended to be in the range of normal base-line values. As is explained below, those cases with little or no change in C′ also tended to have lower parasitemias.

Whole serum complement during primary attacks. Specimens were tested from 22 paroxysms which occurred in 14 patients during their primary attacks. In most instances at least three specimens were taken over a period of 15 h or less, and differences in minimum C′ values before and after peak fever were examined. The percent change in C′ varied from −16 to +8, with one exceptional drop of −38%. The mean change in C′ titer during primary paroxysms was only −2% significantly different from the −23% mean change in C′ titers during paroxysms in relapse ($P = < 0.01$). In Table II more detailed results are presented from representative cases during the primary attack. Whereas it may not be apparent from the examples in Table II, the pre-peak fever C′ levels during primary attacks tended to be higher than normal values, in contrast to the findings during relapse.

The exceptional case with the 38% fall in serum C′ level occurred in an individual during the 8th day of patent infection when he experienced a prolonged fever

TABLE I

C′ Levels Related to Time of Peak Fever and Percent Change in C′ Titer for Representative Cases during Relapses

	Len.*		Sor.		Str.		McG.		McF.		Cros.		Ham.	
	Time	Titer	Time	Titer	Time	Titer	Time	Titer	Time	Titer	Time	Titer	Time	Titer
Base line‡	NA		NA			215		244		208		274	NA	
Time in	P−6½	70	P−4	200	P−26	200	P−4½	154	P−5§	222	P−1½§	202	P−10	167
hours before	P−5½	70	P−2	183	P−½§	163	P−1½§	133	P−3½	233	P	147	P−7§	139
(−) or after	P−4½	67	P−1§	147	P+5½§	149	P+1½§	92	P	164	P+6§	125	P−3	154
(+) peak	P−3½	90	P	112	P+13½	167	P+4	93	P+6§	175	P+17½	182	P	130
fever for	P−2½§	70	P+6§	110			P+7	98	P+16	200			P+4§	50
paroxysm	P−1½	33	P+16	112			P+10	108					P+8	87
studied	P−½§	25					P+16	115						
Difference in titer		−45		−37		−14		−41		−47		−77		−80
Percent change, %		−64		−25		−9		−31		−21		−38		−64

* Since no specimens in this case available after peak fever and because prepeak fever samples were consistently 70 or >, P − 2½ was chosen to calculate change in titer.

‡ C′ level 6–10 days postinfection but before patent parasitemia. NA = no base line available.

§ These are the times selected to calculate change in C′ titer for each case. Titers expressed as $C'H_{50}$/ml of serum.

elevation and maximum parasitemia of 15,600/mm³. This patient was also unique among those studied during a primary attack in that he had developed CF antibody by the 8th day of infection. The findings in this patient are diagrammed in Fig. 1 and will be referred to again in reference to the section on antibody status and parasitemia.

Levels of C4, and other complement components during paroxysms. Serum samples from selected primary and relapse paroxysms were assayed for C4 since low levels of C4 would be expected if complement were utilized via the classical pathway, whereas normal levels of C4 would suggest a reaction involving the alternate pathway. Five paroxysms from primary attacks and eight paroxysms from relapses, including cases both with and without depressions in whole serum C′, were examined for changes in C4. In general, the values for C4 paralleled those of whole serum C′, and those paroxysms which were characterized by impressive reductions in C′ also showed reductions in C4 of roughly the same degree. Titers of C4 are about 10^3 higher than whole serum C′ values and normally range from 100,000 to 200,000 (13). Thus, for example, three paroxysms in relapses which were associated with whole serum C′ depressions of −72, −28, and −62% similarly showed reductions of C4 of −78, −45, and −66%, respectively.

However, in three instances substantial reductions in C4 occurred (−48, −42, and −28%) while whole serum C′ levels were unchanged or only slightly reduced (0, +5, and −11%, respectively). Since no examples of C′ reduction without concomitant change in C4 levels were noted assay of C4 may represent a more sensitive method than whole serum C′ of detecting complement changes in malaria. The pattern of C′ and C4 levels during a primary attack and first relapse in one patient is shown in Fig. 2, and in another case during several paroxysms in relapse in Fig. 3.

Levels of several different complement components,

TABLE II

C′ Levels Related to Time of Peak Fever and Percent Change in C′ Titer for Representative Cases during Primary Attack

	McG.		Mash.		Jon.		Str.		All.		Edge.		McF.	
	Time	Titer	Time	Titer	Time	Titer	Time	Titer	Time	Titer	Time	Titer	Time	Titer
Base line*		244		125		194		215		187		182		208
Time in hours	P−5‡	323	P−12	154	P−1‡	263	P‡	215	P−1‡	215	P−24	256	P−10‡	250
before (−) or	P	308	P−6‡	154	P+5	263	P+6‡	187	P+5‡	233	P−1‡	299	P−4	263
after (+) peak	P+6‡	313	P	156	P+11‡	235	P+12	278	P+11	238	P	285	P+2‡	270
fever for	P+18	323	P+6‡	156	P+17	286	P+18	253	P+17	244	P+6‡	299	P+8	278
paroxysm studied			P+12	156							P+21	270		
Difference in titer		−10		+2		−28		−28		+18		0		+20
Percent change, %		−3		+1		−11		−13		+8		0		+8

* C′ level 6–10 days postinfection but before patent parasitemia.

‡ These are the times selected to calculate change in C′ titer for each case. Titers expressed as $C'H_{50}$/ml of serum.

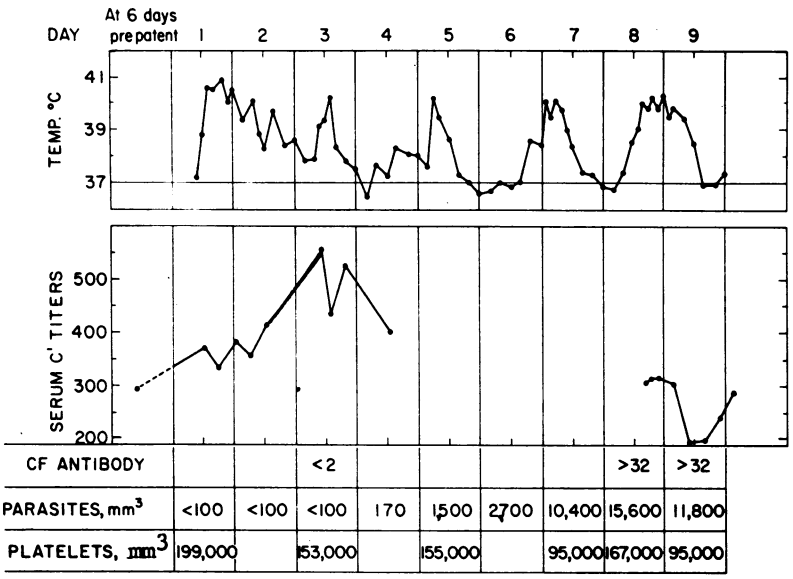

FIGURE 1 Serum C′ levels during primary attack of vivax malaria. Note elevated C′ levels during first 4 days of fever when CF antibody was absent and parasitemia was low. In contrast, C′ fell (−38%) during paroxysm spanning days 8 and 9 when CF antibody was present and parasitemia was high.

including C1, C2, C4, C3, C3 proactivator, and C6 were checked in selected paroxysms. Alterations in C3 and C6 were much less marked than decreases in C1, C2, C4, and whole C′ in four paroxysms during relapse. In two patients there were no changes in C3 and C6. One case did show an 18% drop in C3 and C6 while C4 declined 58%. In a second instance a 23% fall in C3 with even less change in C3 proactivator occurred while whole C′ and early C components decreased quite strikingly. These results are summarized in Table III.

The time-course for alterations in C′ levels in relationship to fever as well as the parasite cycle in the paroxysms of two patients are shown in Figs. 4 and 5. The time interval for schizont rupture can be estimated from several functions, the period required for mature schizonts to disappear after reaching peak num-

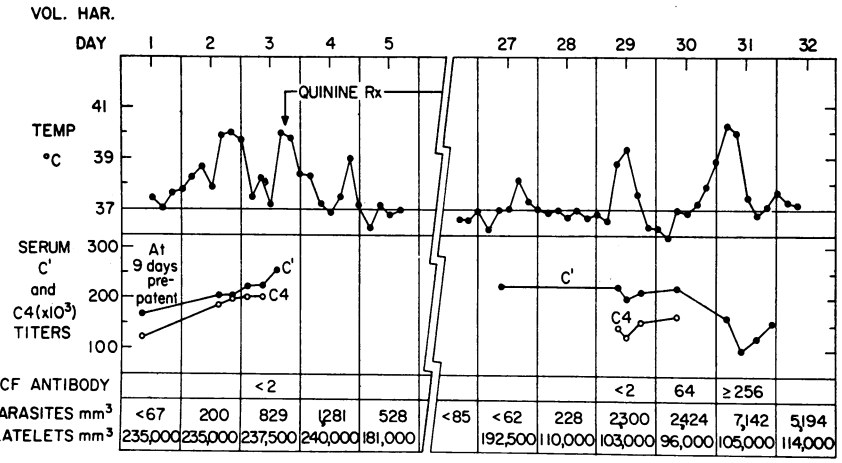

FIGURE 2 C′ and C4 levels in relationship to fever, parasite density, and appearance of CF antibody during primary attack and first relapse. Note stable or rising levels of C′ during primary attack, only a slight drop initially in relapse (−12%) but a striking fall in C′ on the third paroxysm in relapse (−39%).

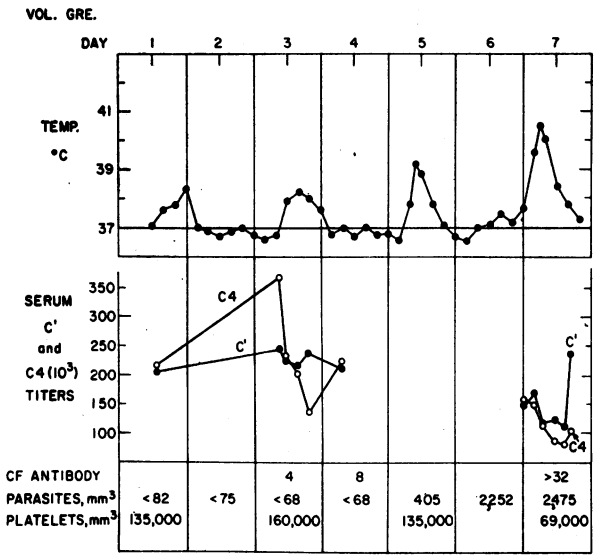

FIGURE 3 C′ and C4 levels in relationship to fever, parasite density, and rising CF antibody level during relapse. Note drop in C4 (−42%) with little change in C′ during second paroxysm, but definite fall in both C′ and C4 during fourth paroxysm (−28 and −45%, respectively).

bers and the period during which ring forms first appeared and reached maximum numbers.

Correlation of complement changes with CF antibody and degree of parasitemia. The relationship of factors such as presence of CF antibody and amount of antigen was next examined. Since *P. cynomolgi* antigen cross-reacts with *P. vivax* antibodies in various serologic tests, including CF (14, 15), and was available, it was used as a CF antigen. Serum samples taken just before and after paroxysms of both primary attacks and relapses were tested for presence of CF antibody. Serum from only 2 of the 21 paroxysms of primary attacks showed presence of CF antibody and in both instances the infection was already in the 8th day or beyond of overt symptoms. One of these (Vol. Chris, see Fig. 1) was the only case exhibiting a significant fall in whole serum C′ during his primary attack. The other case showed no fall in whole serum C′ but did have a drop in C4 titer of 48%. By contrast, CF antibody was present at a dilution of 1:2 or greater at the start of all paroxysms studied during relapse; the lowest serum dilution tested was 1:2. In three instances during relapse the CF titers at onset of paroxysm were low, 1:2, 1:4, and 1:4, but in all other cases the titers ranged from 1:8 to 1:256. CF antibody titers of 1:256 were present in three patients before their relapse paroxysms. It should be noted in all cases during relapse that several days of patent parasitemia and symptoms had already occurred before the paroxysm under study, thus allowing time for a secondary antibody response.

The influence of antigenic load upon likelihood and extent of complement consumption was evaluated by examining degree of parasitemia or parasite density associated with each paroxysm. In general, the parasitemia of *P. vivax* infections in man does not exceed 50,000 parasites per mm³. Peripheral blood parasitemia during the primary attack was low, often ranging from < 100 to 1,500/mm³ before chemotherapy. Only 2 of 14 primary cases reached parasite densities exceeding 10,000/mm³; one was McF. who experienced a paroxysm on the 7th day of patency with parasitemia of 12,870/mm³, and the other was Chris (Fig. 1) who reached a parasite density of 15,600/mm³ on the 8th day of patency.

Parasitemia during paroxysms in relapse, by contrast, tended to be somewhat higher than during the primary attack. For example, by the third or fourth paroxysm parasite densities were often 1,000–10,000/mm³ and one reached a maximum of 26,000/mm³. There was a direct correlation ($r = 0.76$) between the parasitemia and the decrease in C′ levels as is shown in Fig. 6. Only 2 of the 11 paroxysms associated with a significant depression in C′ titer had parasitemias of < 1,000 (450 and 675/mm³, respectively), while the remaining 9 episodes were related to parasitemias ranging from 2,400 to 26,000 per mm³.

Thus the likelihood of C′ depletion occurring during paroxysms of *P. vivax* malaria was positively correlated with presence of CF antibody and the degree of parasitemia.

DISCUSSION

That fever per se was not the responsible factor for C′ changes observed can be adduced from previous work as well as our own observations. C′ activity was actually found to be higher during fever by Schubart et al. (16) in patients with typhoid fever and Ecker, Seifter, Dozois, and Barr (17) found no relationship between fever and C′ titers in different infectious diseases. In our study C′ titers during the primary attack were often elevated and tended to be stable during febrile episodes. Rises in C′ activity have been noted in other infectious diseases and this has been attributed to a nonspecific response in acute inflammatory states (18).

The lack of quantitative and standardized methods for measurement of C′ until more recent times makes interpretation difficult of earliest reports of lowered serum C′ during human malaria (19, 20). Even Radosavljevic's report (4) of apparently impressive reductions in C′ activity during the course of malarial infection included very few observations spanning an entire paroxysm; and he interpreted his results as showing a decrease in C′ before the rise in temperature, which is at variance with our findings. Although Dulaney in 1948 (5) used quantitative assays for C′ in finding generally reduced

TABLE III

Levels in Whole Serum Complement and Components and Percent Change for Each in Selected Vivax Cases during Paroxysms in Relapse

Patient	Hours before or after peak fever	Value of C′ and Components,* *U/ml* or *mg/100 ml*						
		C′	C1	C2	C4	C3	C3PA	C6
Loc.	P − 7	200			123,457			
4th	P − 5		102,997	2,148	115,627	69	11.7	633
Paroxysm	P	77	79,145	533	48,915	60	10.4	629
	P + 4	56	62,656	352	29,343	53	10.2	629
	P + 13		90,588	1,100		68	10.0	633
	P + 15	116	96,395	1,233	51,282		10.1	
	P + 26		104,427	1,736	111,606		10.0	
Δ percent		−72	−39	−83	−75	−23	−14.5	−0.6
Har.	P − ½	165	118,765	1,403	76,923	95	23.5	719
3rd	P + 5½	100	71,923	568	31,746	89	23.2	787
Paroxysm	P + 11½	125	90,909	725	31,746	96	24.5	769
	P + 17½	155	103,093	956	42,194	94	25.6	806
	P + 23½		111,111	1,500	68,278		24.3	
Δ percent		−39	−39	−59	−59	−6.3	−1.3	+7.0
Gre.	P − 5	150			153,846			
4th	P − 3	167			144,928	128		488
Paroxysm	P	115			114,286	123		505
	P + 2					120		505
	P + 4	120			86,957			
	P + 6					124		535
	P + 9	231			101,010			
Δ percent		−20			−40	−6.2		+3.5
Cro.	P − ½	202			126,582	80		813
3rd	P	147			66,667	69		662
Paroxysm	P + 6	125			53,163	66		725
	P + 19½	182			60,606	67		741
Δ percent		−38			−58	−17.5		−18.6

* C1, C2, C4, and C6 estimated by hemolytic assay and expressed as units per milliliter; C3 and C3 proactivator (C3PA) measured by Mancini technique and expressed in mg/100 ml.

C′ levels in patients with induced malaria, her report specifically mentioned that the lowered C′ levels could not be correlated with certain features of the infection, such as the developmental cycle of the parasite, fever, or parasite density. In retrospect, it seems clear that previous studies in human malaria have failed to examine closely and correlate C′ alterations with parasite synchrony, degree of parasitemia, and duration of infection—all of which were found to be important factors affecting C′ levels in this study.

More recently, Fogel, von Doenhoff, Cooper, and Fife (6) reported a convincing reduction in C′ activity during terminal stages of rapidly fatal malarial infections in monkeys, hamsters, and chickens. They also suggested that cyclic changes in total C′ were related to daily merozoite release with *P. knowlesi*. In a companion report, Cooper and Fogel (7) presented evidence that in the monkey with fatal *P. knowlesi* infection the changes in hemolytic activity of whole serum C′ were paralleled by changes in C1a, C2a, and C3 components. Although these authors were able to show a good correlation of C′ depression with degree of parasitemia, they were unable to demonstrate CF antibody in the sera of monkeys with low levels of C′.

While past observations have strongly implicated complement depletion during malaria, its occurrence, exact timing with the parasite cycle, and mechanism have not been clearly defined in human malaria previously. The findings reported here have firmly documented cyclic utilization of C′ during vivax malaria and have identified the principal causative factors. Maximal depression of C′ titers was found between time of peak fever and within 10 h thereafter. While published data for the exact time relationship between rupture of most schizonts

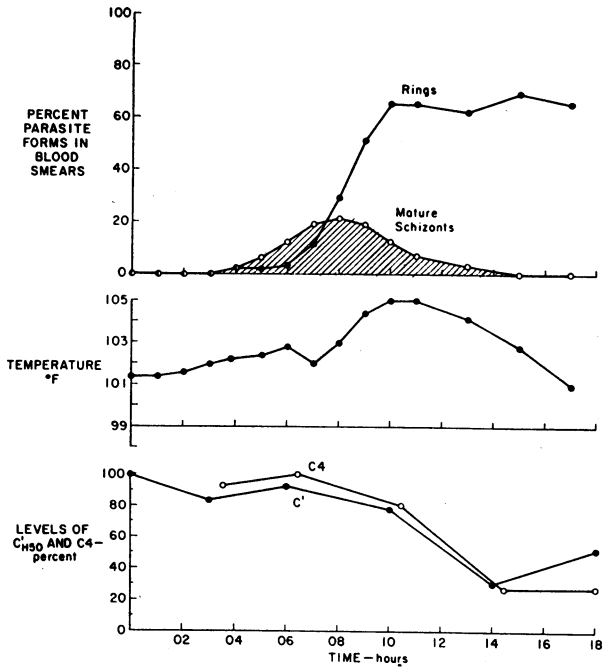

FIGURE 4 Time correlation of parasite cycle with fever and changes in levels of whole serum and C4 complement levels in a vivax paroxysm during relapse.

and maximum fever are not available, our unpublished results indicate that mature schizonts do not disappear from the peripheral circulation until time of peak fever.

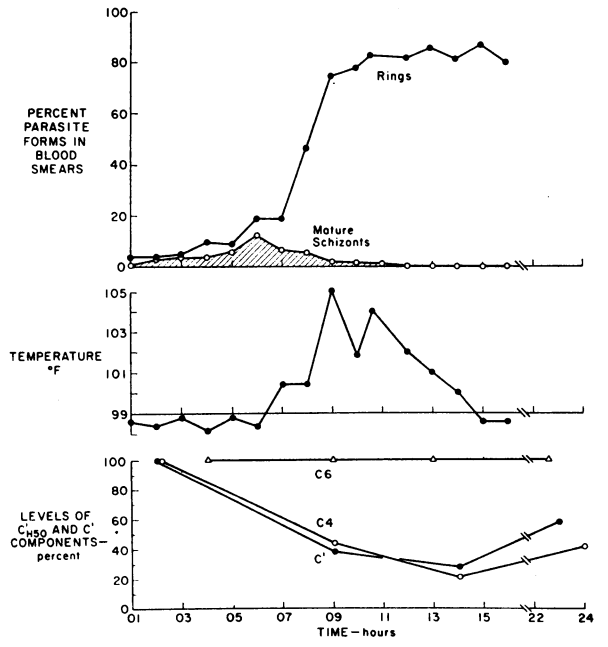

FIGURE 5 Time correlation of parasite cycle with fever and changes in levels of whole serum complement and complement components in a vivax paroxysm during relapse.

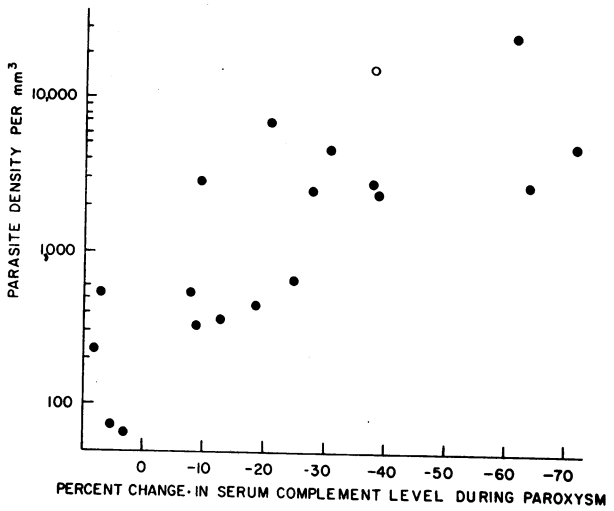

FIGURE 6 Correlation of parasite density with change in serum C′ level during 18 paroxysms in relapse and one paroxysm (open circle) of a primary attack ($r = 0.76$). The latter was one of only two primary paroxysms studied with CF antibody present.

Thus, a close temporal relationship exists between the completion of schizont rupture and minimum C′ levels. The direct correlation of degree of C′ reduction with parasite density also links C′ depletion to schizont rupture and release of merozoites. This was clearly illustrated by several instances in which a fall in C′ occurred only in the latest of a series of paroxysms as parasitemia was increasing in degree. The demonstration of presence of CF antibody in all cases in which C′ depletion occurred suggests that an antigen-antibody reaction is required for C′ depletion. One inconsistency to this hypothesis was the failure of Cooper and Fogel to find CF antibody in their *P. knowlesi*-infected monkeys. Our studies of complement alterations with *P. coatneyi* in monkeys also failed to demonstrate CF antibodies (8). But fluorescent-reacting antibodies were usually present when complement levels fell and inability to show CF antibodies was attributed to development of antigen-antibody complexes since these sera were strongly anticomplementary. Incidentally, demonstration of the presence and nature of the antigen responsible for the utilization of C′ remains to be accomplished.

While the changes in C4 levels indicated that complement was being utilized by the classical pathway, the lack of participation of later complement components, C3 and C6, was unexpected. Determinations of C4 may be more sensitive than whole serum C′ in detecting alterations of complement in malaria since several patients without significant changes in C′ showed 30-50% reductions in C4. Our evidence for lack of change in later components of complement in human malaria is still preliminary since the tests of C3 and C6 levels were done

on only a few patients. However, this same dissociation in depletion of early vs. late components of complement was found in *P. coatneyi* infections of monkeys (9).

The significance of cyclic utilization of C′ during paroxysms remains to be determined. Since a sufficient time for development of CF antibody seemed necessary for C′ depletion to occur, it is unlikely that this type of C′ activation is responsible for fever or other symptoms of malaria which are prominent during the primary attack even when parasitemia is low and CF antibody is not yet present. Since there is experimental evidence for immunologically mediated reactions as a cause of thrombocytopenia in simian malaria (2), the role of C′ in this situation can be suspected. Whereas this was not a specific object of our investigations, platelet counts were generally within a normal range for the 1st wk during primary attacks and tended to drop later in the primary attack or after a few days of relapse as parasitemia rose and CF antibody was present (see Figs. 1, 2, and 3). The role of C′ in functional immunity during malaria cannot be assessed from the results of these studies. In a simian model it was found that destruction of late-acting components of complement (C3–9) by cobra venom factor did not affect level of parasitemia (9). This would suggest that the late components are not necessary for protective immunity. However, the unexpected finding that the reduction in complement levels affects only early components and not late components still leave unanswered the role of complement in protective immunity in vivo. Cohen and Butcher found no effect of C′ on merozoite invasion of red cells in the presence of antibody in vitro as measured by incorporation of radioactive leucine into protein (21). However, there may be limitations to such an indirect in vitro system and participation of C′ in cell-mediated immunity has not yet been adequately studied.

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