STUDIES IN HUMAN MALARIA

XIX. THE COURSE OF THE COMPLEMENT-FIXATION REACTION IN SPOROZOITE-INDUCED ST. ELIZABETH STRAIN VIVAX MALARIA

BY

CHARLES R. REIN,[1] SAMUEL C. BUKANTZ,[1] JOHN F. KENT,[1] W. CLARK COOPER,[2] DAVID S. RUHE [2] AND G. ROBERT COATNEY [2]

Modern interest in the serodiagnosis of malaria received its chief stimulus from the studies of Coggeshall and Eaton (1938) and Eaton and Coggeshall (1939), who demonstrated the appearance of complement-fixing antibodies in the serum of experimentally infected simian and human subjects. Subsequent investigators, dealing with the human infection, have corroborated and amplified these observations in experiments which have employed a wide variety of antigens and test procedures. Their contributions have been summarized in recent reports (Lippincott et al., 1945; Mayer and Heidelberger, 1946) on the serodiagnosis of malaria.

With the recent greatly increased exposure of military personnel in malarious areas, studies to investigate the usefulness of serodiagnostic methods were undertaken at the Army Medical Department Research and Graduate School. Preliminary experiments resulted in the development of precise spectrophotometric methods for the standardization of the hemolytic reaction (Kent et al., 1946; Bukantz et al., 1946; Kent, 1946), and these methods were applied in determining the optimal adjustment of reagents and conditions for the malaria complement-fixation (C–F) test (Bu-

kantz, Kent and Rein, unpublished observations).

The program of testing antimalarials in prisoner volunteers which was iniated in 1944 by the National Institute of Health (Coatney et al., 1948a) has provided exceptional opportunities to evaluate the serologic test and to study the course of the complement-fixation reaction in vivax malaria. The use of experimental sporozoite-induced infections has made it possible to pre-test large groups of selected individuals before inoculation or the administration of protective drugs and to examine successive specimens of serum during all stages of the disease. The current report is based upon serologic results obtained with subjects who were infected with the St. Elizabeth strain of *Plasmodium vivax*. Accounts of their participation in tests of the protective and therapeutic activity of various drugs appear in earlier papers of this series. A separate report (Rein and Kent, 1947) has dealt with the incidence of false positive tests for syphilis in this group of volunteers.

MATERIALS AND METHODS

Subjects for the tests were white male inmate volunteers at the United States Penitentiary, Atlanta, Ga. Only those volunteers in good physical and mental health were accepted. A history of residence in a known malarious area or any suggestion of previous malarial infection

[1] Department of Serology, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D. C.

[2] Subdivision on Malaria, Division of Tropical Diseases, National Institutes of Health, Bethesda, Md.

were grounds for exclusion as was a positive malaria complement-fixation test. Each individual was exposed to the bites of 10 female *Anopheles quadrimaculatus* mosquitoes infected with the St. Elizabeth strain of *P. vivax,* as proved by postprandial examination of the mosquitoes' salivary glands.

Criteria for the diagnosis of malaria were the demonstration of parasites in thick blood smears and the development of a fever of 101 F or more. Thick and thin blood smears, stained with Giemsa stain, were made and examined daily from the tenth day after exposure through the forty-fifth or sixtieth day, then once weekly until the period of late activity [3] was approached, whereupon a twice weekly schedule was initiated. Whenever parasites were found, smears were again made daily. After late attacks, smears were made at least twice weekly until 18 months after exposure.

Records of oral temperature were made twice daily from the tenth through the forty-fifth day after exposure. When a temperature of 101 F or more was recorded, observations were made every 4 hours until completion of therapy in the acute attacks. At later stages in the infection, whenever patent parasitemia was detected, temperature readings were rescheduled.

Successive specimens of blood were drawn from each individual before his inoculation with malaria by mosquito bite, at least 3 times weekly from the 0 day (exposure day) to the sixtieth day after exposure, then once weekly until the first late attack and at least thrice weekly through the period of late

activity. The serums were preserved in tubes containing dried merthiolate sufficient to give a final concentration of about 1 milligram per ml of serum. They were shipped to the Army Medical Department Research and Graduate School, at first by air and later by ordinary mail, after comparative tests showed that the latter conditions did not appreciably affect serologic activity. The specimens were subjected to the malaria complement-fixation test immediately upon arrival. Later, the accumulated serums from sample individuals were retested in single protocols. The results differed but slightly from those obtained with the specimens as they arrived.

Malaria complement-fixation test [4]

The antigen which was finally adopted after extensive trials of various preparations was an alkaline phosphate buffer extract of *Plasmodium knowlesi* prepared by the method of Dulaney and Morrison (1944). This antigen proved at least equal in sensitivity to the best preparation of *Plasmodium gallinaceum,* and the latter was superior to vivax antigen in comparative tests of serums from 53 subjects with sporozoite-induced *P. vivax* and *Plasmodium falciparum* infections.[5] The selected knowlesi antigen could be standardized at an optimal dilution yielding a maximal reaction with

---

[3] Infections caused by the St. Elizabeth strain of *P. vivax* characteristically exhibit an early period of activity, usually beginning about 2 weeks after exposure, and a late period beginning 6 to 12 months after exposure.

[4] The writers are indebted to Mrs. R. G. Coren for valuable technical assistance in conducting the serologic tests.

[5] An initial supply of *P. knowlesi* antigen was kindly provided by Drs. A. D. Dulaney and D. H. Sprunt of the University of Tennessee. Certain *P. gallinaceum* and *P. knowlesi* antigens were prepared through the collaboration of Dr. R. W. Linton and Mr. S. R. Hawkins of the Lederle Laboratories. The vivax antigen was supplied through the courtesy of Drs. M. Heidelberger and M. M. Mayer of Columbia University.

malarial serums and showing minimal anticomplementary properties in critical tests with one 50 per cent unit of complement. While the extract could be shown to contain substances which fixed complement in the presence of strongly reactive syphilitic serum, these substances were lower in concentration than similar components of gallinaceum antigen, and at dilutions optimal for the complement-fixation test did not present a serious diagnostic problem.

Optimal conditions for fixation were established, at 16 to 18 hours, in the refrigerator, at 3 to 6 C. Parallel tests employing fixation for 1 hour in the water bath, at 37 C, were lower in sensitivity, and further incubation at this temperature proved unsatisfactory because of the marked deterioration of complement occurring in reagent controls.

A detailed description of the complement-fixation procedure was prepared by one of us (JFK) for earlier appearance in a manual of malariology (Russell et al., 1946). The test proper employed a total volume of 1.0 ml, the individual reagents being used in 0.2-ml quantities. Serum was heated for 30 minutes in the water bath at 56 C, and 0.2 ml of a 1-in-5 dilution was tested alone and with antigen in the presence of three 50 per cent units of complement. Upon completion of the standard overnight fixation period, a 0.4-ml volume of previously sensitized sheep's erythrocytes (Kent, 1946) was added, and 30 minutes in the water bath at 37 C were allowed for hemolysis. Reactions in tests were read immediately by comparison with standards, representing varying degrees of reaction from 0 to 4 +. A total of 6,604 samples of serum were examined in this manner during the course of the study.

RESULTS

The present report is based upon results obtained during 18 months of observation in a group of 87 subjects who collectively experienced 199 attacks of malaria. To be considered as exhibiting serologic activity, it was required that an individual show reactions of 2 + or greater in at least 2 consecutive serums. Such persistent reactions were associated with all but 4 of the attacks, the exceptions occurring notably in primary attacks.

Patterns of serologic activity

Patterns of serologic activity representative of the entire group are provided by 3 subjects who participated in tests of the protective and therapeutic activity of quinine sulfate (Coatney et al., 1948b). Figure 1 presents their serum reactions in relation to parasitemia and drug administration, the events being located in time according to the day of their occurrence subsequent to the day of exposure to infected mosquitoes. The first example is that of a patient, A-22, who served as a control in the drug experiment. This subject experienced a primary attack of malaria on the thirteenth day after inoculation. Late erythrocytic activity characteristic of the St. Elizabeth strain of *Plasmodium vivax* was first manifest at about 9 months (day 274), when the first relapse developed. Second and third relapses then followed, parasites reappearing at intervals of 24 and 45 days following the discontinuation of therapy in the preceding attack. In the early primary attack, a positive reaction in the complement-fixation test appeared 5 days after the parasitemia and was demonstrable for 39 days. On the other hand, the practice of initiating therapy on the fifth day of parasitemia resulted
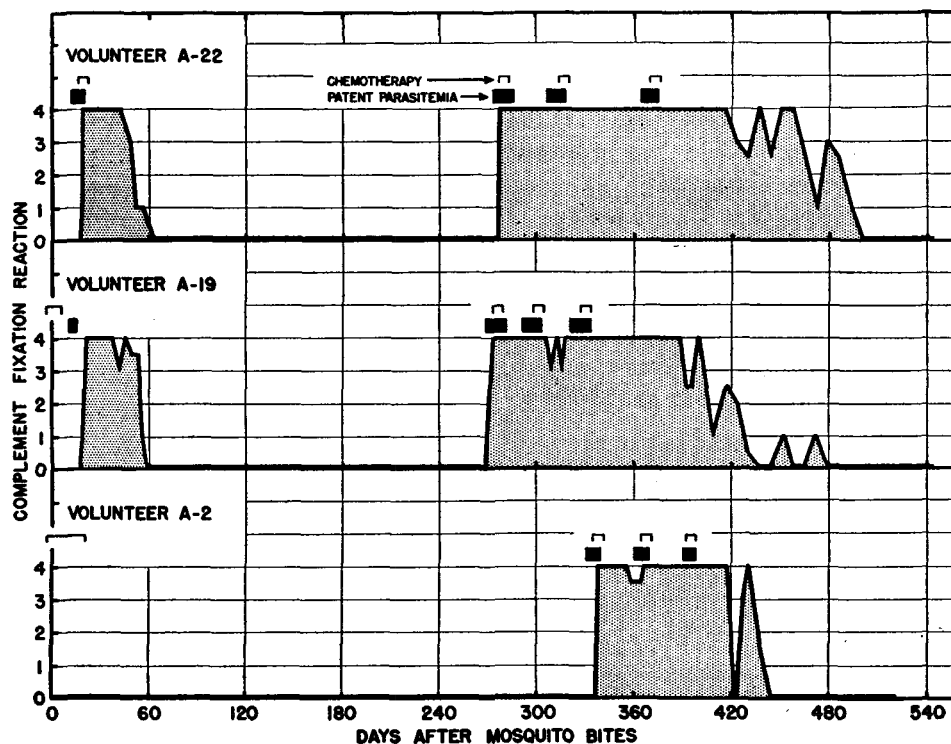
FIGURE 1. Patterns of serologic activity in 3 representative subjects infected
with sporozoite-induced St. Elizabeth strain vivax malaria.

in a disappearance of parasites within 3 days. In the period of late activity, a positive serum reaction reappeared 4 days following patency of the infection and persisted for 216 days, under the stimulus provided by the 3 late relapses. A similar serologic pattern was observed in patient A–19, who participated in the test of the protective activity of quinine sulfate. The drug regimen in this instance proved inadequate for suppression, and a primary attack of malaria occurred on the twelfth day after inoculation. A positive serum reaction appeared 7 days after parasites were demonstrable and persisted for 38 days. The period of late activity, which began on the two hundred seventy-first day was characterized by 3 late relapses, the second and third occurring after intervals of 13 and 18 days, respectively.

In the first of these late attacks, a positive complement-fixation reaction appeared 4 days after demonstrable parasitemia and was continuously evident for 156 days. The third patient, A–2, illustrates the serologic pattern characteristic of subjects in whom early erythrocytic activity was suppressed by schizonticidal drugs. The subject experienced a delayed primary attack on the three hundred and thirty-second day after inoculation, and two successive relapses occurred at 21-day intervals. A positive serologic reaction was demonstrable 7 days after the first patent parasitemia and persisted through the remaining attacks for a period of 99 days.

Certain features of the serologic activity in these 3 individuals were characteristic of the entire group. Positive reactions in the complement-fixation

test for malaria were never encountered in advance of the parasitemia or fever of an attack except in instances when relapses occurred before the reactions due to preceding attacks had subsided. On the other hand, reactivity with plasmodial antigen was consistently of longer duration than the parasitemia, the latter disappearing rapidly following the initiation of therapy.

Time of appearance of serologic activity relative to parasitemia and fever

Data representing the entire group of 87 subjects were examined with respect to the time of appearance of serologic activity relative to parasitema and fever. As a precaution against obscuring possible real differences in the time relations of their serologic response, individuals were grouped according to whether they experienced both early and late erythrocytic activity or late activity only. As indicated earlier, the first of these groups had primary attacks within a few weeks after inoculation, but their relapses were late, appearing at 6 to 12 months after exposure. The second group had no attacks until 6 to 12 months after exposure, but the delayed primary attacks which appeared at the time were followed by early relapses. Separate analyses of the two groups are summarized in table 1. Sections A and B indicate the time relations of the serologic response in successive attacks of malaria.

TABLE 1

Time of appearance and duration of serologic activity in single attacks of sporozoite-induced St. Elizabeth strain vivax malaria

Type of attack*	A				B				C			
	No. of subjects observed	Interval between onset of parasitemia and positive complement fixation (days)			No. of subjects observed	Interval between onset of fever and positive complement fixation (days)			No. of subjects observed	Duration of positive complement fixation (days)		
		Range	Mean	σ		Range	Mean	σ		Range	Mean	σ
I. Subjects experiencing both early and late erythrocytic activity												
Early primary	40	2–14	7.1	2.2	40	1–12	6.0	2.4	39	2–133	41	23.3
First late relapse	38	0–9	3.9	2.0	38	0–7	2.0	2.1	11	31–132	82	36.1
Second late relapse	3	4–21	9.7	—	2	2–18	10.0	—	3	19–47	35	—
Third late relapse	2	3–4	3.5	—	1	1	—	—	2	4–38	21	—
Fourth late relapse	—	—	—	—	—	—	—	—	—	—	—	—
Fifth late relapse	1†	7	—	—	1†	4	—	—	1†	3	—	—
II. Subjects experiencing only late erythrocytic activity												
Late primary	43	0–16	7.3	3.0	43	0–14	6.5	2.9	33	7–193	43	35.0
First late relapse	7	2–9	4.9	2.1	7	0–5	2.1	1.7	5	48–77	62	13.6
Second late relapse	1	3	—	—	1	1	—	—	1	88	—	—

* Early attack = one that occurred within a few weeks after exposure; late attack = one that occurred 6 to 12 months after exposure.

† Serologic activity associated with the fourth late relapse (fifth attack) in this subject was not differentiated by a seronegative interval from the activity accompanying his third late relapse (fourth attack).

The group of 40 individuals who experienced early primary attacks developed positive complement-fixation reactions, on the average, 7.1 days after patent parasitemia and 6.0 days after fever. Corresponding mean values of 7.3 and 6.5 days were obtained for 43 subjects who had delayed primary attacks. Apparently, the long prepatent period characteristic of the second group did not significantly alter the time relations of the initial serologic response.

The most striking feature of these observations was the rapidity with which positive complement-fixation reactions appeared in later attacks. The group of 38 individuals whose first relapses occurred 6 to 12 months after exposure developed positive serologic reactions, on the average, 3.9 days after parasitemia and 2.0 days after fever.[6] These reduced intervals were in marked contrast to the mean time required for the appearance of serologic activity in primary attacks. Seven subjects who had relapses early following delayed primary attacks showed a similar acceleration of the serologic response, but the differences observed in this small group were not significant. Unfortunately, the

_____

6 It should be noted that the schedule adopted in the collection of data (see Materials and methods) placed certain limitations on the accuracy with which the time relations and the duration of serologic activity could be estimated. During early primary attacks, for example, the probable time of appearance of parasites was, on the average, 0.5 days earlier, and of serologic activity, 1.16 days earlier than was recorded. These errors tended to compensate each other so that their combined effect would be a reduction from 7.1 to 6.5 days in the mean interval between parasitic and serologic activity. During the late first relapses, similar compensating errors would have increased the corresponding mean interval from 3.9 to 4.5 days. The probability that the difference between these corrected means could have arisen by chance was estimated to be less than 1 in 1,000.

mass of data relative to serologic activity in subsequent attacks could not be analyzed in similar fashion. A total of 112 relapses was recorded during the study, but in most instances the time relations of the serologic response were obscured by the reactions developed during earlier attacks (see figure 1). It is noteworthy that acceleration of the serologic response occurred as a characteristic of individuals who had responded serologically to a previous stimulating attack. It appeared to be independent of the stage of the infection since it did not characterize delayed primary attacks in subjects who had harbored malarial infections for 6 to 12 months.

Duration of serologic activity

The duration of the serologic activity accompanying single attacks of malaria could be estimated only under conditions where the activity in question was distinguished by seronegative intervals from that of earlier and later attacks. This restriction placed definite limitations upon the number of relapses that could be considered. Examination of the available data revealed no pronounced difference betwen the group of subjects who experienced both early and late erythrocytic activity and that which experienced late activity only. The findings are given in table 1 (section C). In 39 subjects who had early primary attacks, the mean duration of serologic activity was 41 days. Thirty-three individuals whose primary attacks were delayed until 6 to 12 months showed a corresponding mean duration of 43 days. In the first relapses of the respective groups, serologic activity persisted, on the average, for 82 and 62 days, respectively. The range of all observations, however, was wide and the standard deviation high. While the mean values suggested an increase in

the duration of the complement-fixation reaction following second attacks of malaria, the observed differences could not be considered statistically significant. Data relative to the persistence of serologic activity in third and subsequent attacks were too limited for analysis.

During the period of late erythrocytic activity, relapses frequently occurred before the serologic activity due to preceding attacks had subsided. Since the additional stimulus provided by early recurrences tended to prolong seropositivity, reactions observed under these conditions were generally of longer duration than those accompanying single malarial attacks. Reactivity associated with multiple attacks was encountered in 38 individuals. The number of stimulating attacks varied from 2 to 7, and the associated complement-fixation reactions lasted from 49 to 276 days. The mean duration of serologic activity in these subjects was 125 days.

Incidence of patent parasitemia and serologic activity in 87 subjects during 18 months of observation

In an attempt to represent certain features of these observations in graphic form, data relative to parasitic and serologic activity in all 87 individuals were reviewed and the number of subjects exhibiting patent parasitemia on each day after exposure was determined for comparison with the number who showed serologic activity. The comparative totals for 543 days of observation are plotted against time in figure 2.[7] The number of subjects continuing under observation as the studies progressed is plotted for reference. Because of the

----

[7] Since blood for stained smears and serologic tests was not taken daily, it was frequently necessary to interpolate between successive positive reports.

41 subjects who experienced early primary attacks of malaria, the incidence of patent parasitemia rose rapidly to a peak on the eighteenth day after inoculation. It was followed, after an approximate interval of 7 days (compare table 1), by a correspondingly rapid increase in the number of subjects exhibiting a positive complement-fixation reaction. The incidence of patent parasitemia, however, fell quickly after reaching its height, whereas the number of serologically active individuals declined slowly over a period of weeks. Under the optimal conditions imposed by repeated consecutive examinations, the inspection of stained blood smears and the serologic test were approximately equal in diagnostic sensitivity; the serologic test failed to become positive in only one early primary attack. Inspection of the curves indicates that while an arbitrary 50 per cent or more of the infections were detectable for 8 days by the examination of stained blood smears, a similar number were identifiable by serologic test over a period of 40 days.

Of the 87 individuals initially included in these studies, all but one were available for observation during their periods of late erythrocytic activity. These 86 subjects experienced a total of 158 late attacks, of which 46 were primary attacks that had been delayed by the administration of schizonticidal drugs. The latter type of attack accounted for the only serologic failures encountered during the period of late activity; 3 individuals who had delayed primary attacks failed to react serologically although they developed positive complement-fixation reactions during subsequent attacks. Since late activity was encountered at any time from 6 to 12 months after exposure, the corresponding time relations of parasitic and serologic activity are obscured in
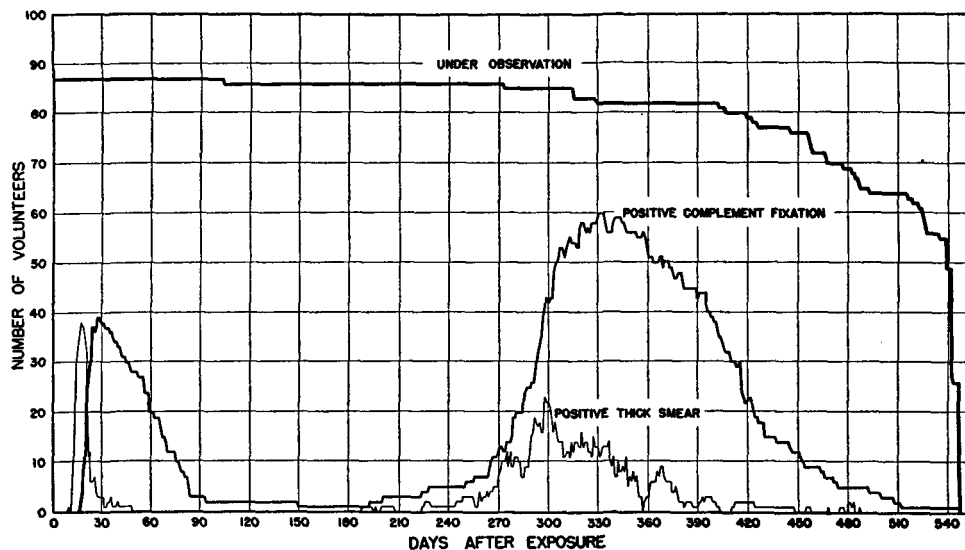
FIGURE 2. Incidence of patent parasitemia and serologic activity during 18 months of obser-
vation in 87 subjects infected with sporozoite-induced St. Elizabeth strain vivax malaria.

the curves of figure 2. It is apparent, however, that at any time during this period the complement-fixation test was superior to the examination of stained blood smears as a method for appraising the malaria experience of the entire group.

DISCUSSION

It appears from the foregoing observations that the complement-fixation test for malaria, at least in infections due to the St. Elizabeth strain of *Plasmodium vivax*, possesses both limitations and advantages as an aid in diagnosis. From the point of view of the establishment of a diagnosis in the individual patient, certain conclusions are apparent. Since serologic activity was never encountered in advance of the parasitemia of a primary attack, the complement-fixation test would appear to be of less value in establishing an early diagnosis of malaria than the examination of stained blood smears. The additional fact that the serologic reaction became negative during the long latent period between early and late erythrocytic activity would make it of little value in predicting relapse. On the other hand, reactivity with plasmodial antigen was consistently of longer duration than the parasitemia. The practice of initiating therapy on the fifth day of patency resulted in a disappearance of parasites within 4 to 5 days, but serologic activity nevertheless persisted. Complement-fixation reactions which developed during primary attacks showed a mean duration of approximately 6 weeks, and reactivity associated with single recurrences appeared equally persistent. This suggested that the complement-fixation reaction might prove of value in identifying recent malarial infections, particularly those rendered parasite-negative by self-medication early in an attack.

When the findings were examined collectively to determine the group characteristics of parasitic and serologic activity during 18 months of observation, it appeared that the complement-fixation test might assume a useful role in mass

diagnosis. As indicated in figure 2, a serologic survey conducted at any time during the period of late erythrocytic activity would have revealed more infections than were detectable by the examination of stained blood smears. During this period individual differences in the pattern of relapse decreased the daily incidence of parasitemia, and the prolonged serologic activity associated with rapidly recurring attacks increased the number of positive complement-fixation reactions that could be demonstrated at a given time. The fact that similar conditions did not obtain during the period of early activity is unimportant from this point of view since exposure would not be simultaneous in a naturally infected group. Moreover, long latency is common for *P. vivax*, and the characteristics of late activity would be the predominant features in an endemic population.

While these observations suggested that the complement-fixation test might prove useful as an accessory examination in survey operations, the present data are too limited in scope to warrant such a recommendation. In the current studies infections were limited to a single strain (St. Elizabeth) of *P. vivax*. Continuing studies include single and combined infections with the St. Elizabeth and Chesson (South Pacific) strains, and the results should provide a better index of the value of the complement-fixation test in diagnosis. It should be emphasized, in any event, that the test for malaria, like other serologic procedures, requires caution in the interpretation of results. Cross-reactions between malarial or syphilitic serums and the corresponding antigens have been measurably decreased by successive improvements in the antigens, but reactions of this type are still encountered.

In our experience, exceptional syphilitic serums, notably those reacting in high dilution with alcoholic tissue extract antigen, have yielded reactions in the range of ± to 2 + with "soluble" knowlesi antigen. However, some indication of the occasional nature of these reactions was provided during the present study by 6 subjects who presented a history of treated syphilis. Of this group, 3 showed partial and 3 complete fixation of complement in successive tests for syphilis. None of these individuals reacted with "soluble" knowlesi antigen during the intervals which preceded their primary attacks, and the reactivity associated with their attacks was indistinguishable in pattern from that observed in patients with uncomplicated malaria. The corresponding cross-reaction between malarial serum and alcoholic tissue extract antigen has been markedly reduced in frequency since the introduction of an optimal cardiolipin antigen.[8] In our studies of sporozoite-induced vivax malaria, only 1 out of 104 infected subjects has developed persistent reactivity in cardiolipin complement-fixation tests (Kent et al., 1948). As a precaution against misinterpretation of results, however, serums submitted for the serodiagnosis of malaria should be tested simultaneously with plasmodial and cardiolipin antigens in a 3-tube complement-fixation test. Under such conditions occasional equivocal results may be resolved by retesting the serum after absorption with floccules of alcoholic tissue-extract antigen (Dulaney and Stratman-Thomas, 1940).

---

[8] The recommended cardiolipin antigen is that described by Maltaner and Maltaner (1945) and contains cardiolipin, 0.0175 per cent; purified lecithin, 0.0875 per cent; and cholesterol, 0.3 per cent in ethyl alcohol. This antigen is diluted 1 in 250 for use under the conditions of the malaria complement-fixation test.

SUMMARY

The course of the complement-fixation reaction with plasmodial antigen was studied for 18 months in each of 87 prisoner volunteers experimentally infected with sporozoites of the St. Elizabeth strain of *Plasmodium vivax*. Positive serologic reactions were associated with all but 4 of their 199 malarial attacks, the exceptions occurring only in primary attacks.

Serologic activity appeared in primary attacks, on the average, 7.2 days after patent parasitemia and 6.2 days after fever. In relapses, the corresponding mean intervals were 4.4 and 2.3 days. The complement-fixation test remained positive for an average of 42 days during primary attacks, and for an average of 125 days during the repeated relapses that characterized late erythrocytic activity. The parasitemia, however, disappeared within 4 to 5 days after the initiation of therapy.

From the point of view of the individual patient, the complement-fixation test for malaria might prove useful in identifying well-developed or recently subsided vivax infections but would be of no value in establishing a diagnosis either early in an attack or during the long latent period between early and late activity. On the other hand, the persistence of serologic activity during recurrent attacks made it possible to detect more infected individuals by serologic test than could be demonstrated by the examination of stained blood smears.

REFERENCES

Bukantz, S. C., Rein, C. R., and Kent, J. F.
1946 Studies in complement fixation. II. Preservation of sheep's blood in citrate dextrose mixtures (modified Alsever's solution) for use in the complement fixation reaction. Jour. Lab. and Clin. Med., *31*: 394–399.

Coatney, G. R., Cooper, W. C., and Ruhe, D. S.
1948a Studies in human malaria. VI. The organization of a program for testing potential antimalarial drugs in prisoner volunteers. Amer. Jour. Hyg., *47*: 113–119.

Coatney, G. R., Cooper, W. C., Ruhe, D. S., Josephson, E. S., Young, M. D., and Burgess, R. W.
1948b Studies in human malaria. VII. The protective and therapeutic action of quinine sulfate against St. Elizabeth strain vivax malaria. Amer. Jour. Hyg., *47*: 120–134.

Coggeshall, L. T., and Eaton, M. D.
1938 The complement fixation reaction in monkey malaria. Jour. Exp. Med., *67*: 871–882.

Dulaney, A. D., and Morrison, D. B.
1944 On the preparation and properties of antigens from *Plasmodium knowlesi*. Amer. Jour. Trop. Med., *24*: 323–326.

Dulaney, A. D., and Stratman-Thomas, W. K.
1940 Specific nature of complement fixing antibody in malaria as demonstrated by absorption tests. Proc. Soc. Exp. Biol. and Med., *44*: 347–349.

Eaton, M. D., and Coggeshall, L. T.
1939 Complement fixation in human malaria with an antigen prepared from the monkey parasite *Plasmodium knowlesi*. Jour. Exp. Med., *69*: 379–398.

Kent, J. F.
1946 An abbreviated spectrophotometric technique for determining the optimal concentration of amboceptor. Jour. Lab. and Clin. Med., *31*: 1270–1277.

Kent, J. F., Bukantz, S. C., and Rein, C. R.
1946 Studies in complement fixation. I. Spectrophotometric titration of complement; construction of graphs for direct determination of the 50 per cent hemolytic unit. Jour. Immunol., *53*: 37–50.

Kent, J. F., Culwell, W. B., Coatney, G. R., and Cooper, W. C.
1948 False positive tests for syphilis: a further study of their incidence in sporozoite-induced vivax malaria. Jour. Lab. and Clin. Med., *33*: 747–752.

Lippincott, S. W., Gordon, H. H., Hesselbrock, W. B., and Marble, A.
  1945 Complement-fixation in human malaria using an antigen prepared from the chicken parasite *Plasmodium gallinaceum.* Jour. Clin. Invest., *24:* 362–371.
Maltaner, E., and Maltaner, F.
  1945 The standardization of the cardiolipin-lecithin-cholesterol antigen in the complement-fixation test for syphilis. Jour. Immunol., *51:* 195–214.

Mayer, M. M., and Heidelberger, M.
  1946 Studies in human malaria. V. Complement-fixation reactions. Jour. Immunol., *54:* 89–102.
Rein, C. R., and Kent, J. F.
  1947 False positive tests for syphilis; a study of their incidence in sporozoite-induced vivax malaria. Jour. Amer. Med. Ass'n, *133:* 1001–1003.
Russell, P. F., West, L. S., and Manwell, R. D.
  1946 Practical Malariology. W. B. Saunders Company, Philadelphia. 684 pp. (See pp. 104–109.)