The Course of the Serologic Tests during Therapeutic Malaria in Patients with Syphilis*

BERNARD I. KAPLAN, M.D., AND I. JAY BRIGHTMAN, M.D., MED.SC.D.

Physician, Sing Sing Prison Hospital, Ossining, N. Y., and Acting Director of Syphilis Control, Westchester County Department of Health; and Consultant, Division of Syphilis Control, New York State Department of Health, Albany, N. Y.

IN connection with investigations on the rôle of malaria in the treatment of syphilis, changes were noted in the quantitative serologic tests during the course of the induced infections. These findings form the basis for this report.

We know of no previous systematic study of the effect of malarial infection upon the quantitative determination of reagin in the serum of patients with syphilis. On the other hand, the development of a false positive serologic test for syphilis during the course of malaria in non-luetics seems to be well established by the recent investigations of Kitchen, Webb, and Kupper,[1] and of Burney, Mays, and Iskrant.[2] The earlier literature on this subject has been reviewed by Hazen and his collaborators.[3]

METHODS AND MATERIAL

All individuals included in this report were studied at Sing Sing Prison Hospital with the exception of 10 patients who were observed in collaboration with Dr. Evan W. Thomas at Bellevue Hospital. Patients with latent or central nervous system syphilis who were to receive malaria therapy were hospitalized on the day of inoculation and were permitted to have 6 to 8 febrile paroxysms. The infection was terminated with either quinine sulfate or atabrine. Intensive arsenotherapy with mapharsen was given either by intravenous drip[4] before or after the anti-malarial agents or by the multiple syringe method[5] which was given concurrently. With the former method the patient received the infusions on 3 consecutive days totalling 600 to 720 mg. of mapharsen while with the latter technic 10 daily injections of 60 mg. of mapharsen were given.

Thirty-three patients with latent or central nervous system syphilis were inoculated with *Plasmodium malariae* and 11 patients were inoculated with *Plasmodium vivax*. The strains employed have been in constant use for several years and have been observed to produce characteristic clinical infections in susceptible individuals.

To control our observations upon the changes in serologic tests in syphilitic individuals during the course of thera-

* From the Syphilis Control Office, New York State Department of Correction and the Department of Medicine, New York University College of Medicine.
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peutic malaria, we are reporting serologic studies made on 50 patients with latent and central nervous system syphilis who were treated with fever induced by intravenous typhoid vaccine combined with intensive mapharsen drip therapy. In addition, we are recording our observations on a group of 25 patients with latent syphilis who received intensive mapharsen drip therapy alone. These 2 groups have been previously described.[4]

An analysis of the patients in these various therapeutic groups showed no significant differences regarding age or color, duration of the disease, previous therapy, and initial serologic titer. In general, the previous treatment was irregular and usually inadequate. The duration of the disease, when known, was evenly distributed between 3 and 20 years in all groups. The age groups would be too small to warrant a breakdown in that regard.

Serologic studies were done 3 times weekly during the course of induced malaria and daily during typhoid vaccine fever and arsenotherapy. All serologic studies were performed in the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. The complement-fixation test, which was done on all specimens, was the quantitative procedure described by Wadsworth, Maltaner, and Maltaner.[6] In addition, quantitative flocculation titers were determined simultaneously on many of the specimens obtained from the malaria treated patients, using both New York State[7] and Kahn[8] procedures. As the results obtained with these two flocculation tests were essentially identical, only the Kahn titers will be reported as this latter test has had more universal usage.

To control the effects of malarial infection upon serologic reactions other than those associated with syphilis, 6 patients were vaccinated with *Eberthella typhosa* antigen a few weeks prior to inoculation with *P. malariae*. Quantitative *E. typhosa* agglutination titers were then determined throughout the course of the malarial infection. The course of the antibodies was controlled by following the titers of 7 vaccinated patients who did not receive malaria therapy.

As this report is concerned only with the serologic changes referable to the actual malarial infection and not to the serologic response noted months later, the results presented include only those occurring during the febrile period and the subsequent week.

RESULTS

Tables 1, 2, and 3 refer to patients receiving therapeutic malaria and record the day following inoculation upon which the first febrile paroxysm occurred, the initial quantitative complement-fixation and Kahn flocculation titers just prior to inoculation, the first day upon which there was noted a tendency of the titer to rise or fall, and the maximal extent of that change. In the case of the complement-fixation test the maximal change is also recorded in terms of the percentage of the initial titer. The Kahn titers are expressed in units.[8] In all but two instances the trends were first noted during the course of the malarial infection but the progression frequently extended into the week following the last paroxysm. The highest or lowest levels reported, therefore may have occurred while the patient was receiving atabrine, quinine sulfate, or intensive arsenotherapy. The negative effect of the latter type of therapy upon the serologic titer is indicated by the control studies.

Of the 23 patients with central nervous system syphilis who were treated with *P. malariae* infection (Table 1), 22 exhibited a progressive fall in complement-fixation titer during the course of the febrile paroxysms. With the one

TABLE 1

The Changes in the Quantitative Serologic Titer in 23 Patients with Central Nervous System Syphilis During the Course of Induced Plasmodium Malariae Infection

		New York State Complement-fixation Titer				*Kahn Flocculation Units*		
				Maximum Changes				
Case Number	*First Paroxysm* *	*Initial Titer*	*Trends Observed* *	*Titer*	*Per cent of Initial Titer*	*Initial Titer*	*Trends Observed* *	*Levels Reached*
1	14	25	Fall — 20	8.9	36	3	Rise — 16 Fall — 23	240 2
2	9	3.6	Fall — 19	0	0			
3	11	36	Fall — 26	8.2	23			
4	5	41	Fall — 9	10	24	20	Rise — 11 Fall — 18	160 3
5	18	18	None	..	..	20	Rise — 27 Fall — 37	200 40
6	9	41	Fall — 20	15	36	80	Fall — 24	4
7	25	12	Fall — 20	1.7	14			
8	27	15	Fall — 34	2.6	17			
9	6	62	Fall — 16	24	38			
10	9	28	Fall — 19	9.3	33			
11	5	17	Fall — 14	7.2	43			
12	6	56	Fall — 17	27	48			
13	10	28	Fall — 28	16	57			
14	18	35	Fall — 27	5.3	15			
15	2	6.6	Fall — 11	2.4	36			
16	9	57	Fall — 24	19	33			
17	8	33	Fall — 27	15	46			
18	6	7.2	Fall — 14	0	0			
19	7	23	Fall — 13	4.7	20			
20	8	30	Fall — 24	5.9	20			
21	18	92	Fall — 28	27	29			
22	26	125	Fall — 35	50	40	160	Rise — 28 Fall — 35	320 80
23	7	7.4	Fall — 10	2.3	31	Neg.	Rise — 10 Fall — 17	20 Neg.

* Noted in days after inoculation with *Plasmodium malariae*

exception noted, the titer in each instance dropped to a level less than 60 per cent of the initial titer and in 7 instances the fall was to 20 per cent or less. Flocculation tests were done on 6 of the 23 patients. Only 1 showed a fall in Kahn units paralleling the drop in complement-fixation titer. The other 5, including the patient who showed no fall in complement-fixation titer, showed a significant rise in the level of Kahn units, although a subsequent fall was noted before the termination of the infection. Of the 10 patients with latent syphilis who were inoculated with *P. malariae* (Table 2), 8 showed a fall in complement-fixation titer to less than 60 per cent of the initial level. In 2 instances this fall was first noted just after, rather than prior

TABLE 2

The Changes in the Quantitative Serologic Titer in 10 Patients with Late Latent Syphilis during the Course of Induced Plasmodium Malariae Infection

		New York State Complement-fixation Titer				*Kahn Flocculation Units*		
				Maximum Changes				
Case Number	*First Paroxysm* *	*Initial Titer*	*Trends Observed* *	*Titer*	*Per cent of Initial Titer*	*Initial Titer*	*Trends Observed* *	*Levels Reached*
24	19	35	Fall — 21	15	43	40	None	..
25	20	33	None	..	..	20	Rise — 33	120
26	18	45	Fall — 18	24	53	20	Rise — 11 Fall — 23	400 4
27	16	20	Fall — 17	4.6	23	20	Rise — 19 Fall — 21	80 20
28	13	42	Fall — 17	14	33	20	Rise — 17 Fall — 19	200 20
29	25	21	Fall — 35 †	10	48	40	Rise — 21 Fall — 31	120 20
30	5	36	None	..	..	40	Rise — 12	320
31	6	22	Fall — 20 †	13	59	Neg.	Rise — 15 Fall — 22 †	80 4
32	8	17	Fall — 15	10	59	2	Rise — 15 Fall — 17	120 3
33	5	17	Fall — 10	8.2	48	40	Rise — 3 Fall — 10	120 20

* Noted in days after inoculation with *Plasmodium malariae*
† Change observed after last malarial paroxysm during anti-malarial therapy

TABLE 3

The Changes in the Quantitative Serologic Titer in 11 Patients with Central Nervous System Syphilis during the Course of Induced Plasmodium Vivax Infection

		New York State Complement-fixation Titer				*Kahn Flocculation Units*		
				Maximum Changes				
Case Number	*First Paroxysm* *	*Initial Titer*	*Trends Observed* *	*Titer*	*Per cent of Initial Titer*	*Initial Titer*	*Trends Observed* *	*Levels Reached*
34	5	10	Rise — 13	98	980			
35	5	15	Fall — 8	5.3	35	20	Rise — 15	400
36	3	27	Rise — 13	50	185	20	Rise — 11	200
37	4	94	Fall — 9 Rise — 12	58 130	62 138	80	Rise — 12	600
38	8	25	Fall — 13	10	40	Neg.	Rise — 11 Fall — 18	400 Neg.
39	7	13	Fall — 9	2.9	22	Neg.	Rise — 11 Fall — 16	240 3
40	6	32	Fall — 11	16	50	80	None	..
41	3	50	None	..	..	120	None	..
42	13	33	None	..	..	120	None	..
43	7	160	Fall — 13	85	53	Neg.	Rise — 13 Fall — 18	600 200
44	6	13	Fall — 13	3.7	28	3	Rise — 13 Fall — 18	40 Neg.

* Noted in days after inoculation with *Plasmodium malariae*

to, the last paroxysm. Kahn tests were done on all of these individuals. Nine exhibited a significant rise in titer, followed in 7 instances by a subsequent fall. The 2 patients in whom a subsequent fall in Kahn units failed to occur were the same individuals who had exhibited no fall in complement-fixation titer.

Thus, of 33 syphilitic patients treated with quartan malaria, 30 exhibited a progressive fall in the complement-fixation titer for syphilis during the induced malarial infection. Of 16 of the 33 on whom quantitative Kahn tests were performed, 14 exhibited an increase in Kahn units, 1 a decrease, and 1 showed no change at all. In 12 of the 14 who showed an increase in Kahn units a subsequent fall occurred before or just after the last malarial paroxysm.

Among the 11 patients with central nervous system syphilis who were treated with *P. vivax* infection (Table 3), 6 showed a progressive fall in complement-fixation to less than 60 per cent of the initial level, and 2 exhibited no significant changes. Three individuals showed a rise in titer although in 1 case this was preceded by a preliminary fall. Flocculation tests were done in 10 of these individuals. An increase in Kahn units was noted in 7 patients, in 4 of whom a subsequent fall occurred. No changes appeared in 3 individuals, 2 of whom had likewise shown no change in complement-fixation titer.

Graphic illustrations of the serologic changes during therapeutic malaria in 4 patients selected from the above groups, together with accompanying descriptive legends, are presented in Figures 1, 2, 3, and 4.

In the control observation group, of 50 patients treated with fever induced by intravenous typhoid vaccine combined with intensive arsenotherapy, only 14 exhibited a fall in complement-fixation titer to 60 per cent or less of the initial level. Seven patients showing a drop in titer were among the 25 with central nervous system syphilis and the remaining 7 were of the 25 with latent syphilis. Of 25 patients with latent syphilis who received only intensive mapharsen therapy by the intravenous drip technic, only 2 exhibited a significant drop in complement-fixation titer during the period of therapy. Increases in complement-fixation titer were noted in only 10 per cent and 8 per cent of these two control groups respectively.

In Table 4 is presented a summary of the serologic changes in the several treatment groups. No differentiation between the patients with latent and central nervous system syphilis is made because of the similarities in the serologic changes as noted above.

The 6 patients in the *P. malariae* group who had been vaccinated with *E. typhosa* antigen a few weeks prior to inoculation showed no significant variation in *E. typhosa* agglutination

TABLE 4

Summary of Major Serologic Trends in Syphilitic Individuals during Various Methods of Therapy

	New York State Complement-fixation Titer					*Kahn Flocculation Units*				
		Fall in Titer		*Rise in Titer*			*Fall in Titer*		*Rise in Titer*	
Method of Therapy	*Patients Tested*	*No.*	*Per cent*	*No.*	*Per cent*	*Patients Tested*	*No.*	*Per cent*	*No.*	*Per cent*
P. malariae infection	33	30	91	0	0	16	1	6	14	88
P. vivax infection	11	6	55	3	27	10	0	0	7	70
Typhoid vaccine fever with intensive arsenotherapy	50	14	28	5	10					
Intensive mapharsen drip therapy	25	2	8	2	8					

FIGURE 1—Serologic changes during induced *Plasmodium malariae* infection in a 46 year old Negro male with central nervous system syphilis, illustrating fall in complement-fixation and Kahn flocculation titers

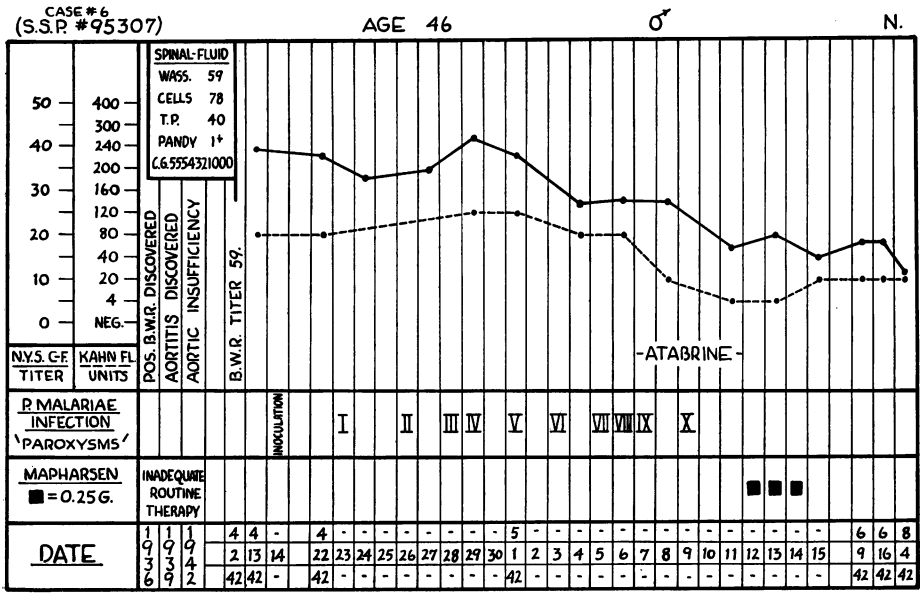


FIGURE 2—Serologic changes during *Plasmodium malariae* infection in a 33 year old white male with central nervous system syphilis, illustrating fall in complement-fixation titer and rise and fall in Kahn flocculation titer

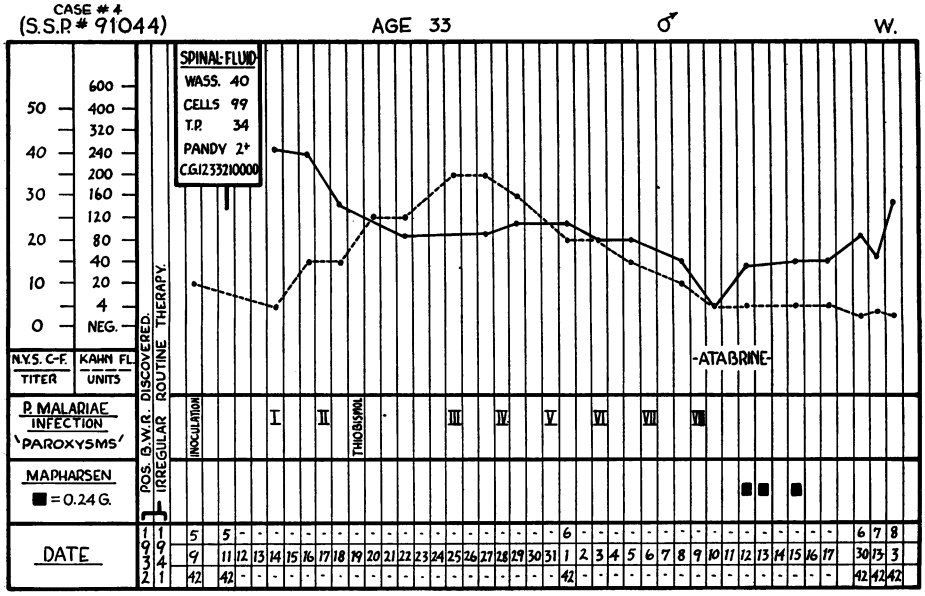


FIGURE 3—Serologic changes during *Plasmodium vivax* infection in a 46 year old white male with central nervous system syphilis, illustrating fall in complement-fixation titer and rise in Kahn flocculation titer

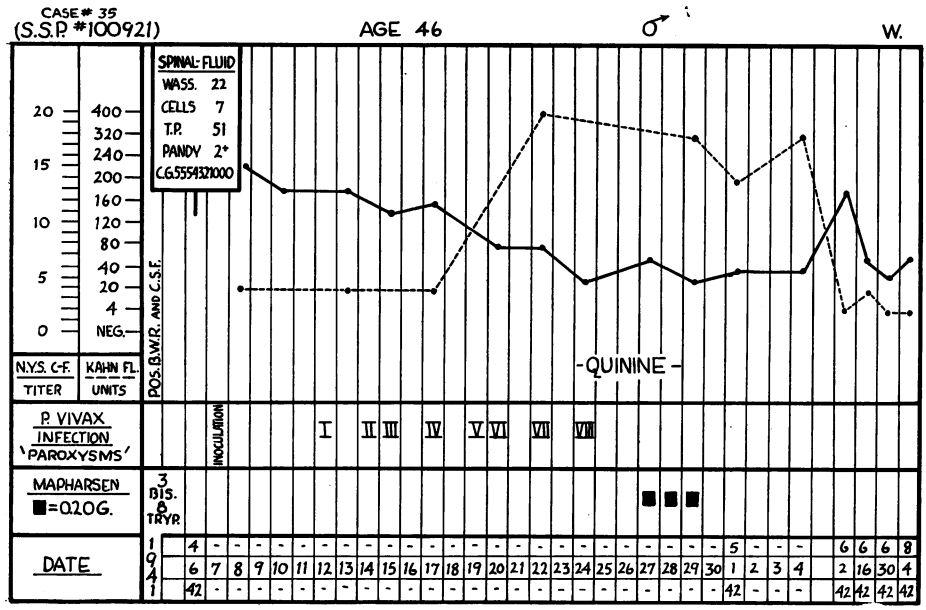
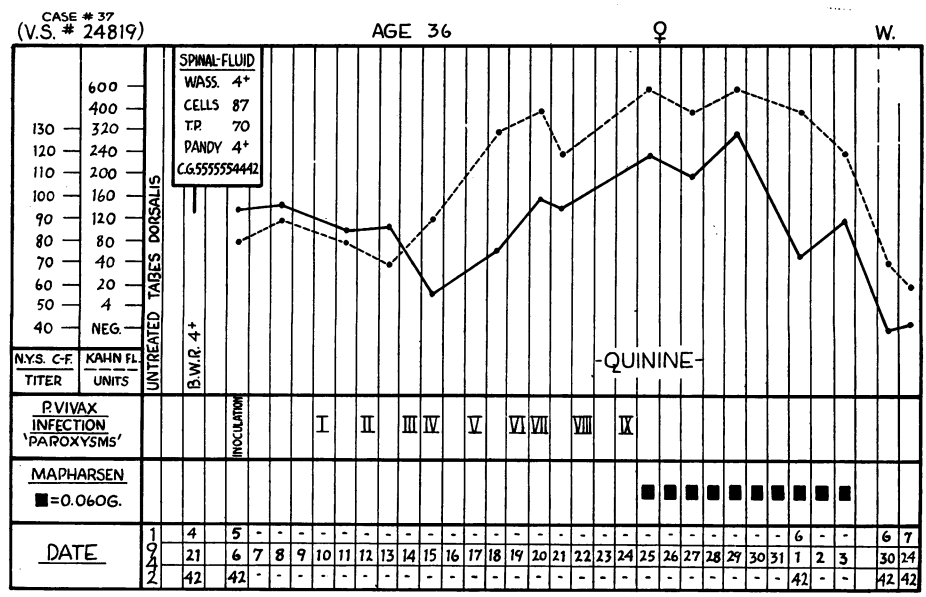


FIGURE 4—Serologic changes during induced Plasmodium *vivax* infection in a 36 year old white female with central nervous system syphilis, illustrating rise in complement-fixation and Kahn flocculation titers after preliminary fall in former, and subsequent fall in both soon after termination of infection



titer during the course of the malarial infection although 5 of the 6 showed considerable falls in the titer of the complement-fixation tests for syphilis.

COMMENTS

The importance of the serologic method in demonstrating alterations in reagin content has previously been demonstrated by Kitchen, Webb, and Kupper, and more strikingly by Burney, Mays, and Iskrant in their studies showing the development of false positive reactions for syphilis in non-luetics during therapeutic malaria. Kitchen and his collaborators noted definite differences in the results of the flocculation and complement-fixation tests done upon individual specimens during the course of either *P. vivax* or *P. falciparum* infections. Burney, Mays, and Iskrant, employing 9 different technics, found marked variations in the frequency of false positive reactions for syphilis during the course of vivax infections, some tests exhibiting few or no positive reactions whereas others showed positive reactions among each of the 11 individuals studied. In the recently published report of the Washington Serology Conference,[9] wherein different laboratories performed individual tests, considerable variations were again noted, the different technics giving positive results in from none to 75 per cent of the 12 cases of malaria tested. Differences in reagin appearance relative to the species of parasite used was noted by Kitchen, Webb, and Kupper, who observed that vivax infections tend to induce a greater proportion of positive serologic results during the course of the disease than did falciparum infections.

Our observations show that definite trends in the quantitative serologic tests occur in syphilitic individuals during the course of induced *P. malariae* infection, but that the direction of the trends is dependent upon the serologic test employed. The different curves presented by the quantitative complement-fixation and flocculation tests for syphilis would appear to be governed by the specificity of the individual methods. The New York State complement-fixation test has previously been shown to possess high specificity. In the Washington Serology Conference referred to above this test revealed only one doubtfully positive reaction among 12 patients with malaria. Our results with quartan malaria seem to confirm this specificity as none of our patients showed an increase in titer over that recorded before inoculation with *P. malariae*. The downward trend of the complement-fixation titer may represent the development of an actual anti-reagin agent or of a factor which interferes with the demonstration of reagin by this method. In either case the depression in titer does not seem permanent as in several patients who have been followed sufficiently long the titer tends to approach its original level after a few months. No patients have been followed long enough to warrant any conclusion regarding a possible relationship between the depression in titer and the therapeutic results.

The most common trend of the Kahn and New York State flocculation tests during quartan malaria was a sharp rise in titer followed by a fall to or below the initial level usually before the termination of the infection. Based upon the work of others [1, 2] the preliminary rise in titer would appear to be consistent with the development of a false positive test for syphilis in nonluetics during malarial fever and would indicate that the two flocculation tests are not quite so specific as the New York State complement-fixation test. In the syphilitic patient this probably represents a false positive reaction superimposed upon a true positive reaction. The tendency of the titer to fall

during the course of the infection differs from the trend noted among non-luetics in whom the newly acquired positivity often persists for 1 or more weeks after the last paroxysm. It is interesting and possibly significant that, of the 14 syphilitic patients who exhibited a rise in flocculation titer, the only 2 who failed to show a subsequent fall had also failed to show a fall in complement-fixation titer.

The trends of the serologic tests noted among the syphilitic patients treated with *P. vivax* infection were not nearly so consistent, although in the majority of patients the findings were similar to those exhibited by the *P. malariae* group. The occurrence of a rise in complement-fixation titer in 3 of the 11 patients seems to stress the importance of the species of parasite upon the serologic response, as Kitchen, Webb, and Kupper have noted (vide supra).

Ellingson and Clark [10] have recently reported experiments demonstrating a rapid reduction in *E. typhosa* antibody titers in typhoid vaccinated rabbits following the induction of severe fever by physical methods. However, fever as a major influence upon the serologic titers in our patients appears to be eliminated by the results of the control groups. Only 28 per cent of the patients treated by fever induced by intravenous typhoid vaccine showed a fall in complement-fixation titer although the febrile bouts in this group were similar in intensity and duration to those observed in the *P. malariae* group in which such a fall was noted in 91 per cent. In addition, at least one instance was observed in which a fall in titer was first noted during the incubation period of quartan malaria prior to the first paroxysm. Arsenotherapy given by the intensive intravenous mapharsen drip appeared to have little effect upon the serologic titers, as significant changes during the course of treatment occurred very infrequently in patients receiving this type of therapy alone.

Kopp [11] has reported the occurrence of marked changes in the plasma proteins in patients with therapeutic malaria. His patients exhibited significant depressions in albumen and usually slight elevations in globulin and fibrinogen, with all factors returning to normal upon termination of the infection. In some cases, the changes were noted before the onset of fever. Fever produced by typhoid vaccine or by the inductotherm caused only slight fluctuations in the plasma protein fractions. Whether these changes in plasma protein bear any relation to the fluctuations in reagin observed in our patients is a matter of speculation.

Our studies on the effect of *P. malariae* infection on induced typhoid antibodies revealed no change in the agglutination titer for *E. typhosa* during the malarial infection, indicating that the effects of this therapeutic method upon the tests for syphilis do not universally extend to other types of serologic reactions.

Interesting in this regard are the studies of Caldwell [12] who, in 1930, noted that the serum of general paralytics who had been treated by induced malaria had a higher titer of agglutinins for cultivated *Treponema pallidum* than the serum of similar but untreated patients. He also noted that an increase in spirocheticidal properties of the serum developed during the course of induced malaria in 6 patients. However, in their studies on the complement-fixation test using cultured spirochetes as antigen, Eagle, Mays, Hogan, and Burney [13] and Kolmer, Kast, and Lynch [14] found that a high percentage of non-syphilitic persons with natural or induced tertian malaria exhibited false positive reactions by this method. Further studies on the relationship between syphilitic reagin and spirochetal

antibodies are necessary before any correlation can be made between Caldwell's findings and our own.

Recently Dulaney, Stratman-Thomas, and Warr [15] have described the development of high complement-fixation titers for malaria in syphilitic individuals treated with induced infection. Because of the importance of exact details concerned in any one serologic test in influencing the resulting titers it is not possible to compare the results of any procedures not done in identical manner. However, it is interesting that the specific or malaria complement-fixing antibody should be shown to increase while the reagin indicated by the New York State complement-fixation tests decreased.

SUMMARY

1. Changes in quantitative serologic tests in syphilitic individuals during the course of therapeutic malaria depend upon the type of test employed and the species of parasite used.

2. During infection induced by inoculation with *Plasmodium malariae* there was a uniform fall in the New York State complement-fixation titer and a preliminary rise and subsequent fall in the Kahn and New York State precipitation titers. During infection induced by inoculation with *Plasmodium vivax* the serologic changes were most often similar but were not so constant.

3. Fever alone did not seem to be a major factor in the production of the serologic changes.

4. No conclusions are warranted regarding any relationship between the changes in serologic titers and potential therapeutic results.

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