

THE SOLUBLE MALARIAL ANTIGEN IN THE SERUM OF
MONKEYS INFECTED WITH PLASMODIUM
KNOWLESI

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When *rhesus* monkeys with acute *Plasmodium knowlesi* infection recover after administration of quinine, complement-fixing antibodies are present at maximum titer in the serum soon after the first acute infection has subsided (1), and protective (2) and agglutinating (3) antibodies are demonstrable somewhat later in the course of the chronic disease. Relapses cause an increase in the titer of complement-fixing antibodies. From previous studies it has been impossible to learn whether the antigens and antibodies concerned in the complement fixation reaction are identical with or different from the antigens and antibodies concerned in active immunity and passive protection.

The results of the present work show that a soluble malarial antigen occurs in the serum of monkeys with acute *Plasmodium knowlesi* infection. This antigen, upon injection into normal monkeys, gives rise to complement-fixing antibodies which are similar to those produced by malarial infection, but no protective or agglutinating antibodies are formed. This paper will describe serological and chemical investigations of the soluble malarial antigen.

Materials and Methods

The sera to be tested for soluble malarial antigen were obtained by allowing the parasitized blood to clot, or by collecting the blood in an equal volume of 2 per cent sodium citrate solution. In either case the serum was separated from the red cells as soon as possible after drawing the blood in order to minimize the possibility of extraction of antigen from the parasites *in vitro*. Chloroform was added as a preservative. The preparations containing sodium citrate were anti-complementary, but these could be made suitable for use in the complement fixation test by dialyzing them for 24 hours and then centrifuging to remove

the precipitated fibrin and globulins. Removal of the fibrin was also accomplished by adding calcium chloride solution in order to bring about clotting of the citrated plasma, shaking with glass beads, and centrifuging. A concentration of about 0.5 per cent calcium chloride was required.

Immune sera were obtained from monkeys with chronic *P. knowlesi* infection. Some of these animals had been repeatedly superinfected with large doses of living parasites. The same sera had been tested by complement fixation with antigens from parasitized red cells or spleens (1) and by agglutination (3).

The method of performing the complement fixation test was identical with the procedure described previously (1), except that serum from monkeys with acute *P. knowlesi* infection was used in place of malarial antigens extracted by saline from parasitized blood cells or spleens. To distinguish this from blood antigen and spleen antigen the antigen-containing serum will be referred to in this paper as serum antigen.

For the immunization of monkeys the citrated plasma from heavily parasitized blood was injected intravenously. In order to exclude the possibility of injecting living parasites, the serum antigen was treated with chloroform, allowed to stand in the ice box for at least 3 days, and centrifuged at 3000 R.P.M. for half an hour before it was injected into the monkeys. After a longer time in the ice box these preparations formed a fibrin precipitate, and it was found necessary to centrifuge them again at 3000 R.P.M.

Detection of the Soluble Malarial Antigen

Strong complement fixation reactions were regularly obtained when the sera of monkeys with severe, acute *P. knowlesi* infection (as antigens) were tested with antiserum from a hyperimmunized monkey. Sera from fourteen monkeys with parasite counts above 500 per 10,000 red blood cells all gave complement fixation up to dilutions of 1:32 to 1:128 with antiserum diluted 1:10. Sera from eight monkeys with chronic malaria and parasite counts below 10 per 10,000 red blood cells gave no complement fixation with the same antiserum. No reaction was obtained when sera from normal monkeys were tested with the immune serum.

The soluble malarial antigen may fail to give a definite fixation of complement with certain immune sera from monkeys with chronic *P. knowlesi* infection. Of fourteen immune sera tested against a single preparation of serum antigen, nine gave strong fixation of complement, two gave weak reactions, and three gave negative reactions. The sera which gave weak or negative reactions with the serum antigen also showed low titers of complement-fixing antibodies when tested

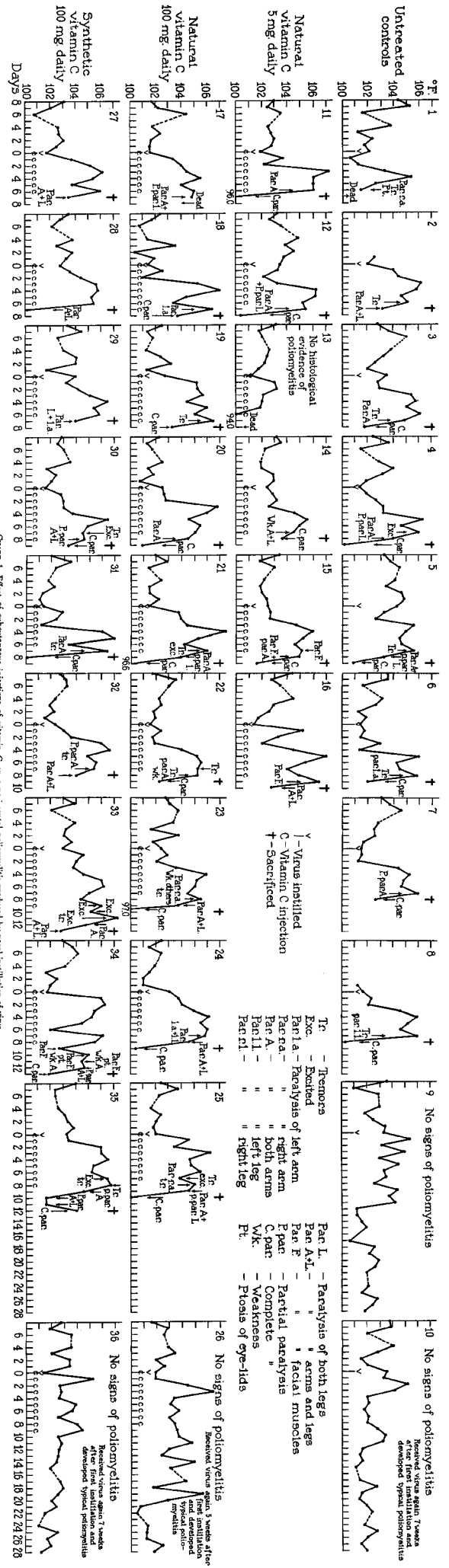


Chart 1. Effect of subcutaneous injections of vitamin C on experimental poliomyelitis produced by nasal instillation of virus.

Received virus again 7 weeks after first instillation and developed typical poliomyelitis

No signs of poliomyelitis between 3 weeks after first instillation and developed typical poliomyelitis

No signs of poliomyelitis Received virus again 7 weeks after first instillation and developed typical poliomyelitis

Tr - Tremors
Exc - Excited
Par l.a - Paralysis of left arm
Par r.a - " " right arm
Par A - " " both arms
Par l.l - " " left leg
Par r.l - " " right leg

Par L. - Paralysis of both legs
Par A.L. - " " arms and legs
Par F. - " " facial muscles
Par P. - Partial paralysis
C. par. - Complete
V/k. - Weakness
Pt. - Ptosis of eye-lids

with antigen prepared from parasitized red cells. Sera from ten normal monkeys gave no fixation of complement with the serum antigen.

Relation of the Soluble Malarial Antigen in the Serum to the Parasite Count

The sera of two monkeys were collected every 24 to 48 hours during acute infection with *P. knowlesi*. The titer of soluble antigen in these sera was determined by complement fixation with an immune serum (diluted 1:10) from a monkey with chronic malaria. The results are

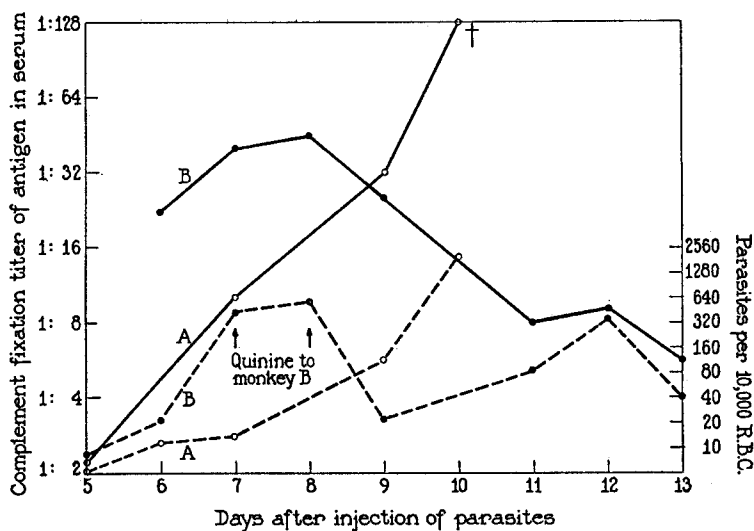


FIG. 1. Relation of soluble malarial antigen to parasite count in acute infection with *P. knowlesi*. Solid lines represent titer of antigen, broken lines parasite count.

presented graphically in Fig. 1. Monkey A received no treatment and died on the 10th day. The rapid rise in the parasite count of this animal was accompanied by a corresponding rise in the titer of soluble antigen between the 5th and 10th days after inoculation. Monkey B was treated by intramuscular injection of 3 cc. of a 3 per cent solution of quinine on the 7th and 8th days and subsequently recovered. The initial rise of the parasite count was accompanied by a rise in the titer of antigen in the serum, and after the injection of quinine the

parasite count and titer of soluble antigen both fell together. However, the secondary rise in the parasite count between the 9th and 12th days was not accompanied by an increase in the amount of soluble antigen in the serum. At this time the monkey was beginning to develop active immunity to the infection.

The disappearance of the soluble malarial antigen from the serum, after the acute infection subsides, is indicated by the results presented in Table I. For example, blood B-1, taken at 6 days, showed a para-

TABLE I
Relation of Titer of Serum Antigen to Parasite Count at Various Times during Plasmodium knowlesi Infection

Blood No.	Time after inoculation <i>days</i>	Parasites per 10,000 red cells	Titer of antigen in serum
A-1	7	16	1:8
A-2	9	121	1:32
B-1	6	21	1:16
3	6	198	1:32
4	14	740	1:64
B-2	13	40	1:4
5	14	510	1:64
F-1	21	664	1:32
6	27	6	Trace
G-1	32	6	0
F-2	33	6	0
7	32	35	Trace
8	62	50	0
G-2	120	335	1:8
G-3	123	150	0

site count of 21 and a titer of antigen of 1:16, while blood B-2, taken from the same monkey at 13 days, had a parasite count of 40 and an antigen titer of only 1:4. When the infection became chronic, no soluble antigen was demonstrable in the serum, although the parasite count was as high as it was at the beginning of the acute infection. This may be seen from a comparison of A-1, A-2, B-1, and 3 with 7, 8, and G-3 in Table I. During a relapse when the parasite count was very high, soluble antigen reappeared in the serum (blood G-1, G-2, G-3), but milder relapses were not accompanied by the appearance of detectable amounts of antigen.

Reaction of Serum Antigen with Malarial Sera from Human Beings

Complement fixation reactions of human malarial sera with an antigen prepared from the parasitized red cells of monkeys dying of acute *P. knowlesi* infection have been described previously (5). The results of performing similar tests with serum antigen in place of antigen from parasitized blood are presented in Table II. The results indicate that the reactions obtained with human sera and serum antigen from monkeys were generally weak or doubtful, although the

TABLE II

Complement Fixation with Human Sera and Antigens from Serum and Parasitized Cells of Monkeys Dying of Plasmodium knowlesi Infection

Serum	Dilution	Serum anti- gen 1:16	Normal mon- key serum 1:16	Antigen from parasitized blood 1:16	Antigen from normal blood 1:4
Malarial human					
Lc	1:4	++	0	++++	+
Rl	1:4	±	0	++++	0
Pn	1:4	0	0	++++	0
Jn	1:4	±	0	++++	0
Rd	1:4	+	0	++++	0
Ir	1:4	+	0	++++	0
Normal human					
Rn	1:4	0	0	0	0
Hn	1:4	0	0	0	0
Er	1:4	0	0	0	0
Dn	1:4	0	0	0	0
Immune monkey	1:4	++++	x	++++	0
Immune monkey	1:4	++++	x	++++	0
Normal monkey	1:4	0	x	0	0

x = not done.

same human sera gave strong reactions with antigen from parasitized blood, and immune monkey sera gave equally strong reactions with both serum antigen and parasite antigen.

Tests for Cross Reactions with Soluble Antigens from Filterable Viruses

Soluble antigens have been described for the viruses of yellow fever (6), vaccinia (7), myxomatosis (8), and influenza (9). Complement-fixing and precipitating antigens are found in the serum during yellow fever infection of monkeys and myxomatosis of rabbits. Although at

first sight it would seem improbable that there are immunological relationships between filterable viruses and malarial parasites, tests for cross reactions were considered desirable because some of these antigens are, perhaps, products of tissue destruction rather than specific components of the virus (6). Sera from monkeys dying of yellow fever and from rabbits dying of myxomatosis were tested for complement fixation with antimalarial monkey sera. Antigens from influenza virus (mouse lung suspension) and lymphocytic choriomeningitis (guinea pig spleen) were also tested for reaction with antimalarial sera. Immune sera against yellow fever, myxomatosis, lymphocytic choriomeningitis, and influenza were tested for cross reactions with malarial antigens prepared from serum and from parasitized blood. In all cases the results were negative.¹ All of these immune sera gave complement fixation with the corresponding specific antigens.

Attempt to Detect Soluble Malarial Antigen in the Urine of Infected Monkeys

Urine was collected from four monkeys dying of *P. knowlesi* infection. One of these animals had a severe hematuria, the urine being dark reddish brown, and two had milder hematuria. These urines together with specimens from normal monkeys were tested for complement fixation with immune serum. The results were completely negative with amounts of 0.1 cc. of undiluted urine. This negative finding indicates that the malarial antigen does not pass the renal glomeruli.

Production of Complement-Fixing Antibodies by the Injection of the Soluble Malarial Antigen into Monkeys

Citrated serum antigen prepared as described in the section on materials and methods was injected intravenously in amounts of 5 cc. into four normal monkeys. Injections were given on 3 successive days; after an interval of 7 to 10 days three more injections were given on successive days. After this, single injections were

¹ Several of the monkeys in the malaria animal room became infected with lymphocytic choriomeningitis in February, 1938. The virus was isolated by Dr. T. F. Francis from animals dying of the disease. The sera of some of the survivors fixed complement with choriomeningitis antigen.

given at intervals of 2 to 3 weeks. The total amounts of serum antigen injected into each of the monkeys were 45, 48, 60, and 70 cc., respectively. During the course of the injections animals were bled at intervals of 1 to 2 weeks and the sera tested for complement fixation with serum antigen and with an antigen prepared from red blood cells parasitized with *P. knowlesi*.

The complement fixation was stronger when the test was done with the antigen prepared from blood than it was with the serum antigen. During the course of immunization two of the monkeys showed maximum complement fixation titers of 1:8 when their sera were tested against the parasitized blood antigen. One monkey showed a maximum titer of 1:16, and the fourth animal a titer of 1:32. These titers are comparable to those of the sera of monkeys with chronic malaria. None of the four animals developed agglutinating antibodies.

The spacing of the injections and the resulting antibody response of one of the monkeys are illustrated in Fig. 2. The antibody titers were determined by complement fixation with an antigen prepared from parasitized blood. 1 week after the first series of three injections there was a slight rise in the titer of antibodies. A specimen of serum taken 1 day after the second series of three injections showed a "negative phase" or decrease in titer. This was followed by a marked rise in the titer of antibodies which reached a maximum after 3 weeks. The titer was maintained near 1:32 by single injections given at intervals of 2 weeks.

The effect of injecting serum antigen intravenously into monkeys with chronic *P. knowlesi* infection is shown in Fig. 3. Blood smears were made daily from these animals and were examined for parasites. Monkey E showed no parasites during the course of the study. The serum of this animal showed a falling complement fixation titer before the injection of serum antigen on Apr. 5. 1 day after the series of three injections there was a definite negative phase, and this was followed by a sharp rise in the titer of complement-fixing antibodies. 2 weeks later the titer had fallen to a level near that which preceded the injection of antigen. Monkey D had parasitic relapses on Mar. 4 and 5 and on Mar. 27 and 28. As has been reported for other experiments in a previous paper (1), relapses are followed by a rise in the titer of complement-fixing antibodies. Monkey D received serum

SOLUBLE MALARIAL ANTIGEN

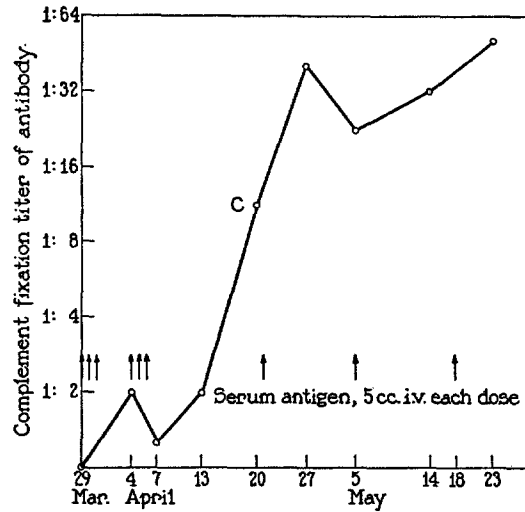


FIG. 2. Production of complement-fixing antibody by injection of malarial antigen.

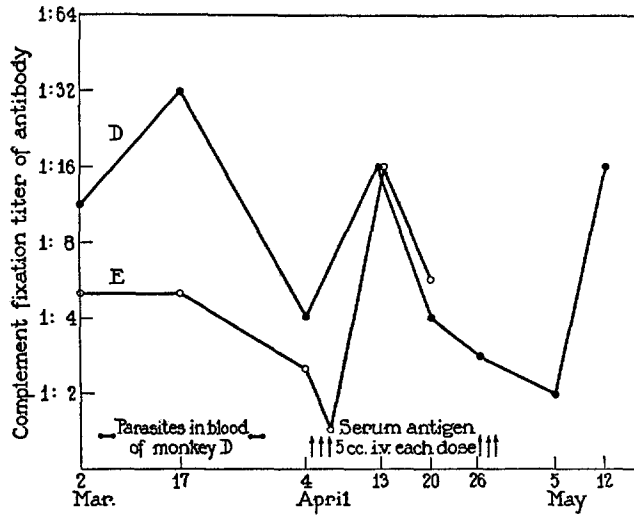


FIG. 3. Stimulation of antibody response by injection of malarial antigen into monkeys with chronic infection.

antigen intravenously on Apr. 5, 6, and 7, and again on Apr. 26, 27, and 28. Since the first series of injections was given shortly after a relapse, the subsequent rise in titer of complement-fixing antibodies

could not be attributed to the injection of antigen. However, no relapses preceded or followed the second series of injections of serum antigen. The resulting rise in titer of complement-fixing antibodies was similar to that produced by a relapse.

Tests for Immunity to Infection

The four normal monkeys which had been injected with soluble malarial antigen were inoculated intraperitoneally with 10,000 living *P. knowlesi* 1 week after a series of injections lasting 6 weeks to 2 months. All four animals became infected after an incubation time which was not appreciably longer than that observed with normal monkeys. One of the animals died with a low parasite count during the early stages of infection and, at autopsy, showed extensive tuberculosis. Two monkeys died with heavily parasitized blood 12 days after inoculation, and the course of the disease in these animals did not differ from that in normal monkeys. The fourth animal, whose serum had a relatively high complement fixation titer of 1:32, developed a very heavy infection but recovered and has remained well for 6 months.

The significance of these results will be considered in the Discussion.

Stability of Serum Antigen

When serum containing the soluble malarial antigen is allowed to stand in the refrigerator, the titer of the antigen gradually falls off over a period of several months. The antigen prepared from parasitized blood also shows a similar lack of stability in a saline solution. Neither antigen is destroyed by heating to 56°C., but a temperature of 70°C. inactivates them. The serum antigen is lost as a result of repeated chemical manipulation. Acids and alkalis destroy both serum antigen and the antigen obtained from parasites. Both may be precipitated by 0.66 saturated ammonium sulfate without appreciable alteration.

Fractionation of Serum Containing the Soluble Malarial Antigen

The serum used in these experiments was obtained from citrated blood by centrifuging down the red cells and defibrinating with calcium chloride solution as described in the section on materials and methods.

Preliminary experiments showed that part of the antigen was carried

down in the globulin fraction precipitated by half-saturated ammonium sulfate, and part remained in the albumin fraction constituting the supernatant. Antigen was precipitated from the globulin fraction by carbon dioxide, but no antigen was precipitated from the albumin fraction by similar treatment with carbon dioxide. Attempts to fractionate the serum antigen by acid precipitation were unsuccessful, the antigen being lost when the pH was brought below 5.5. The studies were, therefore, confined to fractionation by dialysis, ammonium sulfate, and carbon dioxide. During dialysis and fractionation with ammonium sulfate the pH was maintained at all times as near 7.0 as possible.

Experiment 1.—Defibrinated serum giving complement fixation at 1:64 was dialyzed overnight in a cellophane bag. Carbon dioxide was then passed through the dialyzed preparation until no further precipitation occurred. The precipitated globulins were dissolved in saline. This was designated fraction 1*a*. To the supernatant from fraction 1*a* solid ammonium sulfate was added to produce a 0.66 saturated solution. Practically all of the antigen was contained in this precipitate. No antigen could be detected in the supernatant after dialysis. This was discarded. The precipitate produced by 0.66 saturated ammonium sulfate was dissolved in water, the resulting solution dialyzed, and a further portion of the globulins precipitated by carbon dioxide. The redissolved precipitate was designated fraction 2*a*. The supernatant from fraction 2*a* was then fractionated with ammonium sulfate by collecting the precipitates produced successively at 0.33, 0.40, 0.52, and 0.63 of saturation, dissolving in water, and dialyzing the resulting solutions of protein. These fractions were designated 3*a*, 4*a*, 5*a*, and 6*a*, respectively. In all of these fractionation experiments the precipitates were redissolved so as to make a volume of solution approximately half that of the solution from which the precipitate was obtained.

The six fractions just described were tested for complement fixation with the immune monkey serum that was used in detection of the malarial antigen in the whole serum. The results are presented in Table III. The first globulin fraction (1*a*), precipitated by dialysis and carbon dioxide, was very anticomplementary. The second globulin fraction (2*a*), precipitated by carbon dioxide, and the fraction (3*a*), precipitated by 0.33 saturated ammonium sulfate but not precipitated by carbon dioxide, both contained considerable amounts of antigen. The fractions (4*a* and 5*a*), precipitated at 0.40 and 0.52 saturated ammonium sulfate, contained practically no antigen, while the albumin fraction (6*a*) contained the remainder of the antigen in higher concentration than any of the other fractions.

Experiment 2.—The serum giving complement fixation at 1:64 was dialyzed in a cellophane bag as in Experiment 1, but the resulting precipitate of globulin was collected without treatment with carbon dioxide. The globulin precipitate

dissolved in saline was designated fraction 1*b*. The supernatant from fraction 1*b* was then fractionated with ammonium sulfate, the precipitates being collected successively from 0.44, 0.54, and 0.66 saturated solution. The last precipitate of albumin was dissolved in water, dialyzed, and tested for complement-fixing antigen without further treatment. This was designated fraction 10*b*. The fractions precipitated at 0.44 and 0.54 saturated ammonium sulfate were dialyzed separately. Each formed, upon dialysis, a precipitate of globulins, and as these precipitates were found to contain much anticomplementary material, they were

TABLE III
Fractionation of Monkey Serum Containing Soluble Malarial Antigen

Fraction No.	Method of precipitation	Highest dilution giving fixation of complement with immune serum	Highest dilution which was anti-complementary
1 <i>a</i>	Dialysis and CO ₂	1:64	1:64
2 <i>a</i>	0.66 saturated ammonium sulfate and CO ₂	1:32	0
3 <i>a</i>	0.33 saturated ammonium sulfate	1:16	0
4 <i>a</i>	0.40 saturated ammonium sulfate	0	0
5 <i>a</i>	0.52 saturated ammonium sulfate	Trace undiluted	0
6 <i>a</i>	0.66 saturated ammonium sulfate	1:64	0
1 <i>b</i>	Dialysis whole serum	1:64	1:32
2 <i>b</i>	CO ₂ , 0.33 saturated ammonium sulfate, dialysis	1:64	1:8
3 <i>b</i>	CO ₂ and 0.33 saturated ammonium sulfate	1:8	1:8
4 <i>b</i>	CO ₂ and 0.50 saturated ammonium sulfate	1:32	1:16
5 <i>b</i>	CO ₂ and 0.66 saturated ammonium sulfate	1:8	0
6 <i>b</i>	0.44, then 0.33 saturated ammonium sulfate	1:32	1:8
7 <i>b</i>	0.54, then 0.45 saturated ammonium sulfate	1:4	0
8 <i>b</i>	0.54 saturated ammonium sulfate twice	1:4	0
9 <i>b</i>	0.54, then 0.66 saturated ammonium sulfate	1:32	0
10 <i>b</i>	0.66 saturated ammonium sulfate	1:32	0

For a detailed description of the preparation of the various fractions, see text.
0 = no fixation of complement or no anticomplementary effect.

discarded. Carbon dioxide was then passed through the two dialyzed solutions of proteins (precipitated at 0.44 and 0.54 saturated ammonium sulfate); the two redissolved carbon dioxide precipitates were combined; and the two supernatants were combined.

The carbon dioxide precipitates were refractionated with ammonium sulfate at 0.33, 0.50, and 0.66 of saturation. After being dissolved each of the three fractions was dialyzed. The fraction precipitated at 0.33 saturated ammonium sulfate formed, upon dialysis, a globulin precipitate which was designated frac-

tion *2b*. The supernatant from fraction *2b* was designated fraction *3b*. The other two ammonium sulfate fractions of the carbon dioxide precipitates were designated *4b* and *5b*, respectively.

The supernatants remaining after the precipitation with carbon dioxide were also refractionated. Four fractions were successively precipitated with 0.33, 0.45, 0.54, and 0.66 saturated ammonium sulfate, redissolved, and dialyzed to remove ammonium sulfate. These fractions were designated *6b*, *7b*, *8b*, and *9b*, respectively.

The results of complement fixation tests with the ten fractions just described are presented in Table III. As in the previous experiment the globulin precipitate (*1b*) obtained by dialysis of the whole serum was anticomplementary; the albumin fraction (*10b*) contained much of the antigen. Upon refractionation of those portions of the serum precipitated by 0.44 to 0.54 saturated ammonium sulfate and carbon dioxide, a distribution of antigen between the first globulin fraction (*2b*) and the last fraction (*5b*) was obtained, while the intermediate fractions *3b* and *4b* contained little or no antigen. A similar result was obtained by refractionating the serum protein that was precipitated by 0.44 to 0.54 saturated ammonium sulfate but not precipitated by carbon dioxide. Antigen was present in the globulin fraction *6b* and the albumin fraction *9b*, but relatively little antigen was found in the fractions precipitated by 0.44 and 0.54 ammonium sulfate (*7b* and *8b*).

Since antigen precipitable only by 0.66 saturated ammonium sulfate was obtained by refractionation of the globulin precipitates, the results suggest that the antigen found at first in the globulin fraction was merely adsorbed to the protein precipitated by carbon dioxide, or 0.33 saturated ammonium sulfate. However, since a complete separation of antigen from certain of the globulins was not accomplished by refractionation, we cannot exclude the possibility that there is a second antigen having the properties of a globulin which is distinct from the one found in the albumin fraction.

Experiments with High Speed Centrifugation

It was considered possible that the antigen found in the globulin fraction might represent a colloidal suspension of cellular fragments which were carried down mechanically in the first globulin precipi-

tates. In order to test this possibility the following experiment was performed.

Two samples (A and B) of globulins precipitated by carbon dioxide and containing soluble malarial antigen were dialyzed, and the resulting opalescent suspensions were centrifuged at 11,000 R.P.M. for 2 hours. Some protein was thrown down, but all of the antigen remained in the supernatants which were almost entirely clear. The supernatants were then centrifuged at 27,300 R.P.M. for 3 hours in celluloid tubes, each of which contained 6 cc. The vacuum type air-driven centrifuge described by Bauer and Pickels (4) was used. The contents of the tubes were then divided into three levels for preparation A and six levels

TABLE IV
Sedimentation of Soluble Malarial Antigen by High Speed Centrifugation of the Globulin Fraction of Monkey Serum

Preparation tested	Highest dilution giving fixation of complement with immune serum	Highest dilution which was anti-complementary
Original, sample A	1:16	1:4
Upper 2 cc. sample A, centrifuged	1:4	0
Middle 2 cc. " " "	1:8	0
Lower 2 cc. and sediment of sample A, centrifuged	1:32	1:8
Original sample B	1:16	1:8
1st cc. (top) of sample B, centrifuged	Trace	0
2nd cc. of sample B, centrifuged	1:4	0
3rd cc. " " "	1:6	0
4th cc. " " "	1:6	0
5th cc. " " "	1:6	0
6th cc. " " "	1:16	1:8
Sediment of " " "	1:32	1:16

and sediment for preparation B, using a sampler according to the method of Hughes, Pickels, and Horsfall (10). The centrifuged fractions were then tested for protein, anticomplementary material, and complement-fixing antigen.

The results are presented in Table IV. Most of the anticomplementary material was thrown down by the high speed centrifugation, and the lower third of the liquid and the sediment contained two or three times as much protein as the upper two-thirds. A considerable proportion of the antigen remained in the supernatant, and the titer was not reduced much below that of the original material when allowance was made for the anticomplementary properties of the latter.

There was no striking concentration of antigen in the lower levels of the tubes and the sediment, but these portions were more anticomplementary than the original material.

DISCUSSION

The soluble malarial antigen, which is found in considerable quantity in the serum of monkeys during acute infection with *Plasmodium knowlesi*, apparently is concerned, at least in part, in the production of specific complement-fixing antibodies. These antibodies reach a high titer 1 to 2 weeks after the peak of the parasite count. The appearance of complement-fixing antibodies in the serum is accompanied by disappearance of the soluble antigen. The changes in complement fixation titer which occur during acute and chronic *P. knowlesi* infection may be reproduced by intravenous injection into monkeys of parasite-free serum containing the malarial antigen.

Immunization of normal monkeys with the soluble malarial antigen does not give rise to protective antibodies, agglutinating antibodies, or to an efficient active immunity. One of the four *rhesus* monkeys immunized in this way survived after a severe infection, but this result is not considered significant. *Rhesus* monkeys have occasionally survived without treatment after acute infection with *P. knowlesi*. Observations on several hundred monkeys indicate that the mortality of the untreated infection is over 95 per cent. In chronic *P. knowlesi* infection the production of agglutinating and protective antibodies seems to be a slower process than the production of complement-fixing antibodies, and the antigens concerned are apparently distinct from the complement-fixing antigen.

The first results of chemical fractionation of serum containing malarial antigen suggested that two antigens may be present. One of these was precipitated with the albumin fraction. The second was found in a portion of the globulin fraction. The anticomplementary properties of these globulin fractions and the instability of the antigen make it difficult to characterize the substance accurately. Refractionation experiments indicate that at least a part of the antigen found in the globulin fraction was adsorbed to the precipitates. The antigen found in the serum is both chemically and serologically similar to an

antigen extracted from the parasitized red cells. However, the fact that sera from certain *rhesus* monkeys and from human beings infected with *P. knowlesi* give weak or negative reactions with the serum antigen and strong reactions with comparable dilutions of the antigen prepared from parasitized red cells indicates that an additional complement-fixing antigen is present in the parasitized cells.

In its chemical properties the malarial antigen found in the serum of monkeys infected with *P. knowlesi* resembles the antigen found by Hughes (6) in the serum of monkeys dying of yellow fever. The two antigens do not, however, exhibit any serological cross reactions. On the basis of the present evidence, it is impossible to decide whether the malarial antigen concerned in complement fixation is a product of destruction of red cells or a component of the parasite. The fact that the antigen does not produce active immunity to malarial infection is not to be considered evidence of its origin from the red cells rather than the parasites. Certain bacterial antigens, such as the H antigen of the typhoid bacillus, may play an important part in serological reaction but have little to do with protection against infection.

SUMMARY

A soluble malarial antigen which fixes complement with immune serum is found in the serum of monkeys infected with *Plasmodium knowlesi*.

The amount of antigen in the serum is related to the parasite count during the acute phase of the infection. The antigen is not excreted in the urine.

Intravenous injection into normal monkeys of serum containing the antigen stimulates the production of specific complement-fixing antibodies which react with antigens extracted from parasitized cells, as well as with the antigen present in serum obtained during the acute phase of infection.

Monkeys immunized with serum antigen apparently possess very little or no immunity to infection.

The soluble malarial antigen is labile to acids and alkalies, is not destroyed by a temperature of 56°C., and is precipitated, for the most part, in the albumin fraction of the serum by ammonium sulfate.

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