100 days, respectively.

Rickettsemia. R. quintana appeared in the blood of all the 7 monkeys. The rickettsemia lasted from 10 to 82 days as estimated from the length of the interval between the first and the last positive louse test performed on each monkey. In 6 monkeys the first blood withdrawal for the louse test was performed 6, 7, 8, 8, 9, and 16 days, respectively, after the monkeys had been inoculated. All tests were positive. The blood of the 7th monkey was negative on the 7th day, positive on the 16th. The degree of the rickettsemia could be estimated approximately in each monkey from the percentage of lice which became positive in each consecutive test. In the lice which had been inoculated rectally, the proportion of positive lice reached from 39 to 100%, respectively. Materially the same percentages of positive lice were obtained with intracelomic inoculation. Of the 3 batches of lice which were fed, each on a different monkey, one batch consisted of freshly hatched larvae, the other 2 consisted of adult females. Ten per cent of the larvae became infected with *R. quintana,* whereas of the females 79 and 100%, respectively, became infected. The last positive louse test performed on each monkey scored 4 to 24% of infected lice. Considering the fact that the lice received either a single intrarectal or intracelomic injection of blood diluted 1:5, or were allowed only a single feeding, the high percentage of positive takes indicated a considerable degree of rickettsemia in all our monkeys. After a single feeding on a human case of trench fever, the percentage of lice showing rickettsiae, in our experience, may be much lower than that observed in these 7 *rhesus* monkeys.

Summary. Rhesus monkeys were successfully inoculated with one or another of 4 strains of *R. quintana.* Several of the animals showed a long-lasting rickettsemia. As is the case in trench fever of man, the infection in some of the monkeys remained subclinical. There was no correlation between clinical manifestations and the degree of rickettsemia. The *rhesus* monkey is a satisfactory animal for the experimental study of trench fever.

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CH Hemagglutinogen in Primates.* (20465)

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Since the Ch factor was first reported(1), our efforts have been directed toward the development of high titer anti-Ch serum devoid of any heterospecific agglutinins and to extend our observations to individuals of all blood groups rather than limiting our studies to individuals of blood group O, as was previously done.

Preparation of anti-Ch serum. Prior to starting the immunization, the sera from young, male rabbits were tested for their normal heterospecific titer using OMN Rh-positive washed human erythrocytes for the determination. In no case were rabbits employed which showed the presence of species agglutinins in a titer exceeding 1-32. Following such screening, immunization with washed chimpanzee cells was started. Two male

* Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the results of his own study and do not necessarily reflect the opinion of the Veterans Administration.

TABLE I. Protocol Illustrating Absorption of Anti-CH Serum by Means of Human Cell Stroma and Packed Washed Human Cells.

Date	Absorption No.	Cell suspension*	Dilution of serum after addition of cell suspension (figure below each dilution indicates degree of agglutination) 1–2	1–4	1–8	1–16	1–32	1–64	1–128	1–256	1–512	1–1024	1–2048	1–4096	1–8192	1–16384
5–13	Unabsorbed	Group A	4	4	4	4	4	4	4	4	3+	3	—	—	—	—
		B	4	4	4	4	4	4	4	4	3+	2	—	—	—	—
		O	4	4	4	4	4	4	4	4	3+	1	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	4	4	4	4	4	2	1+	—
	Serum absorbed with equivalent vol of stroma from groups A and B human erythrocytes for 3 hr at 30°C, followed by 15 hr at 5°C															
5–14	1	Group A	4	4	4	4	4	4	4	3+	1	—	—	—	—	—
		B	4	4	4	4	4	4	4	4	2	—	—	—	—	—
		O	4	4	4	4	4	4	4	3+	1+	—	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	4	4	4	4	4	2	1	—
	Serum reabsorbed with equivalent vol of fresh stroma for 2 hr at 30°C with constant agitation															
5–14	2	Group A	4	4	4	4	4	4	3+	3	1	—	—	—	—	—
		B	4	4	4	4	4	4	4	3	1+	—	—	—	—	—
		O	4	4	4	4	4	4	4	3+	1+	—	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	4	4	4	3+	1	—	—	—
	Serum reabsorbed with 2 equivalent vol of fresh stroma for 3 hr at 37°C with frequent agitation															
5–15	3	Group A	4	4	4	4	4	4	3	2+	1	—	—	—	—	—
		B	4	4	4	4	4	4	3+	3	1	—	—	—	—	—
		O	4	4	4	4	4	4	4	3+	1	—	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	4	4	3	2+	1+	—	—	—
	Serum reabsorbed with equal vol of packed washed ABMN, Rh_0-positive human erythrocytes for 2 hr at 33°C with frequent agitation															
5–20	4	Group A	4	4	4	4	4	2+	1	—	—	—	—	—	—	—
		B	4	4	4	4	4	4	2+	1+	—	—	—	—	—	—
		O	4	4	4	4	4	4	3+	3	—	—	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	4	4	3	2	1	—	—	—
	Serum reabsorbed with half vol of packed washed ABMN, Rh_0-positive human erythrocytes for 3 hr at 30°C without agitation															
5–20	5	Group A	4	4	4	3+	3	2+	—	—	—	—	—	—	—	—
		B	4	4	4	4	4	3+	2+	1+	—	—	—	—	—	—
		O	4	4	4	4	3+	3	—	—	—	—	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	4	4	3	2	1	—	—	—
	Serum reabsorbed with half vol of packed washed OMN, Rh_0-positive human erythrocytes for 2 hr at 30°C with frequent agitation															
5–21	6	Group A	4	4	4	3+	2+	1	—	—	—	—	—	—	—	—
		B	4	4	4	2+	1+	1	—	—	—	—	—	—	—	—
		O	4	4	4	4	3	2	—	—	—	—	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	4	3+	3	1	—	—	—	—

chimpanzees (*Pan troglodytes verus*) both OM Rh_0hr' were employed. These were selected from the group recently described by the author(2).

Immunization was started by the intravenous injection of a 20% sterile saline suspension of washed (3 times) chimpanzee cells. The usual amount initially injected was 2 ml. In general, these were followed in one week by a second intravenous injection of a 20% cell suspension from the same chimpanzees. Seven days following the second injection, blood was withdrawn from the rabbits and the serum, after inactivation, was checked for titer against OMN Rh-positive human cells. Generally, we found that the titer was now 1-256. Similar injections at weekly intervals were usually given intraperitoneally and continued until a titer of 1-1024 to 1-2048 was obtained when checked separately against a 2% suspension of washed A, B, O, and AB human cells. When such titers were reached, blood from all rabbits was withdrawn from the heart. This was allowed to clot and clear serum obtained by centrifugation. If any hemolysis was present, the serum was rejected.

Absorption technic. After inactivation at 56°C for 30 minutes, the sera were pooled prior to absorption. In order to reduce hemolysis which usually developed after repeated absorptions of high titer sera with packed human erythrocytes, our initial step was to absorb the serum with given volumes of stroma† from group A and B, Rh_0-positive human erythrocytes. Each volume of stroma represents an equivalent volume of mixed A and B cells. Table I shows the results obtained throughout 3 absorptions with varying amounts of stroma and varying conditions of absorption. It should be noted that the third stroma absorption failed to alter the titer of the serum and only slightly modified the degree of agglutination of cells in any of the serum dilutions employed. Further absorptions were carried out with washed packed human cells, as described in Table I,

† The stroma used in these absorptions was generously donated by the Medical Research Foundation of Dade County, Miami, Fla. The preparation of this has, to date, not been published.

TABLE I (continued).

Date	Absorption No.	Cell suspension*	Dilution of serum after addition of cell suspension (figure below each dilution indicates degree of agglutination) 1–2	1–4	1–8	1–16	1–32	1–64	1–128	1–256	1–512	1–1024	1–2048	1–4096	1–8192	1–16384
Serum reabsorbed with half vol of packed washed OMN, Rh_0-positive human erythrocytes (Negro donor) for 2 hr at 30°C with constant agitation																
5–22	7	Group A	4	4	4	3+	1	—	—	—	—	—	—	—	—	—
		B	4	4	4	3+	1+	—	—	—	—	—	—	—	—	—
		O	4	4	4	3+	1+	—	—	—	—	—	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	4	3	1	—	—	—	—	—
Serum reabsorbed with half vol of packed washed OMN, Rh_0-positive human erythrocytes for 2 hr at 31°C with frequent agitation																
5–28	8	Group A	3+	2	1	—	—	—	—	—	—	—	—	—	—	—
		B	3+	2	1	—	—	—	—	—	—	—	—	—	—	—
		O	3+	2+	1	—	—	—	—	—	—	—	—	—	—	—
		Chimpanzee†	4	4	4	4	4	4	3+	3	1	—	—	—	—	—

* Mixture of washed cells from 2 or more group A, B or O humans. All mixtures contained cells of M and N types and all were Rh_0-positive.
† Washed cell suspensions.

until no further reduction was noted in the reaction with chimpanzee cells. We consider the values, after a total of 8 absorptions, represent the titer of the specific Ch antibody.

It was possible, by an additional absorption with OMN Rh-positive human cells, to remove the antibody so that the serum failed to react with human cells of any blood group. However, absolutely no alteration resulted in the degree of agglutination of chimpanzee cells in any serum dilution and the end-point remained constant (1-512). After the titration of absorbed serum, the undiluted serum was filtered through a Seitz filter and to this filtrate was added Merthiolate (1-10,000). Tests showed that such treatment failed to alter further the titer of the serum.

Slide test. Using the serum absorbed to the point where agglutination of human cells still occurred in a titer of 1-8, a screening test was performed. For this purpose, 3 or 4 drops of finger blood were placed on a slide to which one drop of the anti-Ch serum was added. These were mixed by means of a wooden applicator to cover an area of approximately two-thirds that of the slide. The slides were then placed on a viewing box at a temperature of 42°-45°C and tilted back and forth for a period of 2 minutes. The interval after which agglutination was first apparent was reported as the "appearance time" and the relative degree of agglutination was recorded at the end of 2 minutes.

Results of slide tests. Before starting studies on human bloods, we tested the blood of several chimpanzees, spider monkeys (*Ateles geoffroyi*), rabbits and mice. Two male chimpanzees (*Pan troglodytes verus*) and one female pygmy chimpanzee (*Pan paniscus*) were tested. One of the males was OM Rh_0hr', the other male and the female were AM Rh_0hr'. All 3 chimpanzees' blood reacted with the anti-Ch serum in 5 seconds and all showed a complete agglutination reaction with this serum within 2 minutes.

The bloods of spider monkeys, rabbits and mice failed to react with the anti-Ch serum within the 2-minute period.

We first tested 57 Negroes by this method. Of these, 55 (96.5%) were Ch-positive and 2 (3.5%) were found to be Ch-negative. We

TABLE II. Slide and Tube Agglutination Reactions of Chimpanzee and Human Erythrocytes with Anti-CH Serum, with Special Reference to Family of C.W. (No. 3–7).

No.	Description	Sex	Race	Blood group	Slide test: App. time	Slide test: React. time after 2 min.	Tube test: Serum dilutions after addition of cell suspensions 1–2	1–4	1–8	1–16	1–32	1–64	1–128	1–256	1–512	1–1024
	Chimpanzee	♂		AM†	5 sec.	++++	4	4	4	4	4	4	3+	3	1	-
	,,	♂		OM†	5	++++	4	4	4	4	4	4	3+	2+	1	-
	,,	♀		AM†	5	++++	4	4	4	4	4	4	3+	3	1	-
1	M.B.	♀	N	BM†	18	++	3+	2+	1+	—	—	—	—	—	—	—
2	G.F.	♂	N	OM†	—	—	2+	1+	—	—	—	—	—	—	—	—
3	C.W.	♂	N	ON†	—	—	1+	1	—	—	—	—	—	—	—	—
4	,, sister	♀	N	NR	NR	NR	1	—	—	—	—	—	—	—	—	—
5	,, ,,	♀	N	NR	NR	NR	2	2	—	—	—	—	—	—	—	—
6	,, father	♂	N	NR	NR	NR	3+	3+	3	2+	1	—	—	—	—	—
7	,, mother	♀	N	NR	NR	NR	—	—	—	—	—	—	—	—	—	—
8	A.B.	♂	W	OMN†	15 sec.	++	3+	3+	2	—	—	—	—	—	—	—
9	J.C.	♂	W	AN†	—	—	1	—	—	—	—	—	—	—	—	—

* Pygmy chimpanzee.
† Rh-positive when tested with anti-Rh_0 (anti-D) serum.
NR = Not run.

were forced to limit our studies on Whites to 35 as our available supply of absorbed serum was practically depleted. Of these, 34 (97.1%) were Ch-positive and one (2.9%) was Ch-negative. The ABO group and the MN and Rh types in both Whites and Negroes were distributed within the limits of recognized percentages.

Tube test. To study further the individuals whose blood gave a negative slide test, we prepared serial dilutions of anti-Ch serum with saline. To each dilution, an equal volume of a 2% washed cell suspension of the individual's blood was added, the contents agitated and then centrifuged at 2,000 r.p.m. for 3 minutes. The degree of agglutination was then determined by examination over a viewing box.

Results of tube tests. Tube tests with chimpanzee blood and anti-Ch serum confirmed results of slide tests. Regardless of species, sex or blood group, the degree of agglutination in each serum dilution is almost identical and the end-point (1-512) is the same in each.

We selected at random from the slide tests, one White and one Negro who showed a minimal appearance time and a maximal agglutination after 2 minutes. Reference to Table II shows their reaction in the tube test as compared to the chimpanzee and those individuals who were negative on the slide test. Since the families of Nos. 2 and 9 (Table II) were not available, our studies in the genetics of this factor were limited to the family of No. 3. The blood of his mother, father and 2 sisters were checked and the results are shown in Table II. From these data, we assume the father to be homozygous Ch-positive while the mother was homozygous Ch-negative; whereas the children were heterozygous Ch-positive, thereby strongly suggesting that the Ch agglutinogen is a transmitted Mendelian dominant.

Summary. In our first paper describing this factor, the serum was diluted 1-10 prior to a single absorption with washed packed OMN Rh-positive human erythrocytes. Our present serum has been absorbed, undiluted, under varying conditions, 3 times with cell stroma from group A and B human erythrocytes and 5 times with washed packed human erythrocytes of groups A, B, and O. It is of interest that all chimpanzees of both group A and group O and of both sexes, reacted in almost an identical manner when tested by either the slide or tube test method. One of these was a pygmy chimpanzee (*Pan paniscus*). After 6 absorptions (Table I) no alteration in the titer occurred with chimpanzee cells. Although our studies are not complete, we have presented the following facts: That an anti-chimpanzee (anti-Ch) serum of high antibody titer has been developed by repeated injections of washed chimpanzee cells into rabbits. That the great majority of both Whites and Negroes contain the Ch agglutinogen, in varying degrees, in or on their erythrocytes. Individuals do exist, however, whose erythrocytes completely lack this hemagglutinogen. That, as in all other human blood factors, the Ch factor is an apparently inherited characteristic, transmitted as a Mendelian dominant and that it is not associated with any of the recognized hemagglutinogens of man. We are continuing studies on a relatively large number of individuals of different races in an effort to further determine the racial distribution of this factor and to enable us to work out, in a more comprehensive manner, the genetics of our problem.

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