DEMONSTRATION OF PASSIVE IMMUNITY IN EXPERIMENTAL MONKEY MALARIA

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It is generally accepted that malaria infections confer a certain degree of immunity on their hosts upon recovery. It has not been clearly shown, however, that the serum of an animal after recovery from the acute attack contains immune substances which are capable of exerting a protective action when injected into a normal animal.

In the literature there are conflicting reports concerning the protective property of serum taken from animals or man suffering from chronic malaria. Taliaferro and Taliaferro (1), working with canaries, and Nauck and Malamos (2), using monkeys, reported their failure to produce passive immunity with malaria immune serum, irrespective as to whether it was given before, at the time, or after inoculation of the parasite. However, Findlay and Brown (3), working with canaries, believed that they could demonstrate some protective action if they used sufficiently large doses of immune serum in conjunction with a very small infective dose of parasites. In induced human malaria Kauders (4) felt that convalescent serum had some protective action as well as a beneficial therapeutic effect. Sotiriadès (5) reported clinical improvement in seven malaria-infected paretics after intramuscular injections of 20 cc. of whole blood taken from individuals with chronic malaria; in one case the injections of serum were combined with administration of small amounts of quinine.

When *Plasmodium knowlesi* is injected into *Macacus rhesus* the resultant infection almost invariably terminates in death. Mulligan and Sinton (6) reported that in a series of over 120 monkeys infected with this parasite only one monkey recovered spontaneously from the initial attack. In our experience there was only one survival among 70 infections. An infection with this parasite, however, may be converted into a chronic state by administering antimalarial drugs early in the course of the disease.

Materials and Methods

Two species of monkey plasmodia were represented in this series of experiments. One was a strain of *Plasmodium knowlesi* (Sinton and Mulligan) obtained through

the courtesy of Sir S. Rickard Christophers at the London School of Hygiene and Tropical Medicine in 1934. The other was a strain of *Plasmodium inui* (Halberstadter and Prowazek) isolated in this laboratory in 1933 from a naturally infected *Macacus cynomolgus*. *Macacus rhesus* monkeys were used exclusively in these experiments. They were infected by intravenous injection of citrated blood containing a known number of parasites.

The so called immune serum used in the protection tests described below was collected and pooled from animals with chronic infections of varying duration. The time interval between quinine treatment and bleeding varied from 15 to 190 days, or an average of 41 days. In the pool there was included serum of only one monkey which had been given atabrine instead of quinine. This animal had received one dose of the drug and it was not bled until 28 days after treatment. All bleedings were done under ether anesthesia.

In the early experiments the donor animals were exsanguinated. Later, however, a more economical procedure was adopted by bleeding the animals at infrequent intervals without complete exsanguination. It was found that an amount up to 50 cc. of blood could be obtained without sacrificing the animal. Red blood cell counts were made on all monkeys which had been bled and it was found possible to repeat the bleedings at intervals of approximately 3 weeks. The injections of serum were generally given intraperitoneally, with the exception of one experiment in which some of the serum was injected by the intramuscular route.

After injection of the parasites, blood examinations were made daily on all animals to acquire information concerning the progress and intensity of the infection. The number of parasites found in the blood of infected monkeys, as shown in Table I, is based upon the number of parasitized cells per 10,000 normal red blood corpuscles. In Charts 1 to 5 inclusive the *P. knowlesi* counts were plotted on an arithmetical scale with the ordinates compressed and in Chart 6 the *P. inui* counts were plotted on an unmodified arithmetical scale. When estimating the number of parasites to be used for an inoculation, a simultaneous red cell and parasite count was made on the donor animal. From the data thus obtained the volume of infected blood necessary to contain the desired number of parasites was computed.

EXPERIMENTAL

Experiment 1.—A normal *rhesus* monkey, No. 1, was given 22 cc. of pooled serum intraperitoneally from animals with chronic infection. 3 hours later this animal and another normal monkey, No. 2, which served as control, were each given 1,600,000 parasites intravenously. The course of infection in these two animals is shown in Chart 1 and Table I. It will be noted that the control monkey died on the 8th day after inoculation, while in monkey 1 the course was considerably prolonged and the animal did not succumb to the infection until the 13th day.

Experiment 2.—In this experiment a normal monkey, No. 3, was given 15 cc. of pooled serum from monkeys having chronic infection. Another animal, No. 4,

received a similar amount of normal monkey serum to serve as a control. 3 hours later these two animals and also an additional monkey, No. 5, were each inoculated with 4,500,000 parasites. Immediately following the injection of parasites monkey 3 was given an additional 15 cc. of pooled serum from monkeys with chronic infection, and similar amounts were injected daily for the following 4 days. Monkey 4 received equivalent amounts of normal serum at the same time intervals. The results of this experiment are shown in Chart 2 and Table I. It will be seen that monkey 3, which received pooled serum from monkeys with chronic infection, survived. Monkey 4, which was given normal serum, died on the 8th day, and monkey 5, which received no serum, died on the 12th day following inoculation.

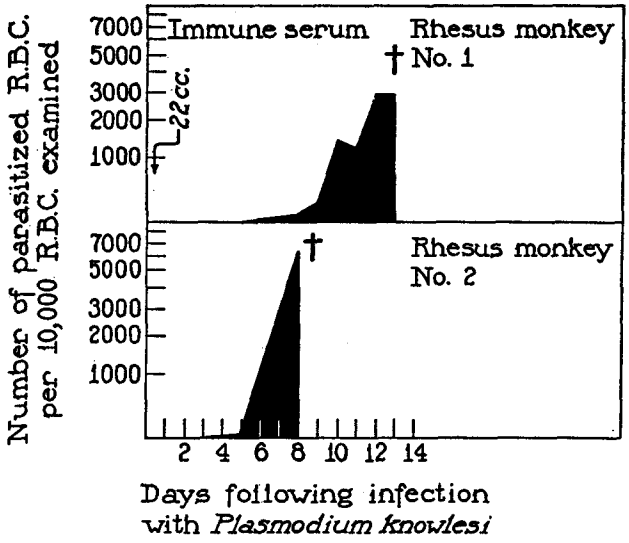

CHART 1

Experiment 3.—The foregoing experiment was repeated along similar lines, except that a different pool of immune serum was used and a normal serum control was not included. Two normal monkeys, Nos. 6 and 7, were each inoculated with 6,000,000 parasites. Monkey 6 had received 25 cc. of immune serum 3 hours prior to the injection of parasites and was given additional injections of immune serum at intervals, as used for monkey 3 in the preceding experiment, and it survived (Chart 3 and Table I). The control animal, No. 7, died on the 7th day after inoculation with the parasites.

Since it was evident from Experiments 2 and 3 that the serum from monkeys harboring chronic malarial infections is capable of conveying

TAB

Summary of Protection Test Results in Monkeys, Showing the Amount of Immune Serum Injected at Daily Parasite Count in the Circula

Serial No. of monkey	No. of parasites injected	3 hrs. before		Simultaneous		1		2		3		4		5		6		7		Day
		Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	
		cc.		*cc.*		*cc.*		*cc.*		*cc.*		*cc.*		*cc.*		*cc.*		*cc.*		
1	1,600,000	22					0		0		0		0		3		3		29	
2 c	1,600,000						0		0		4		4		29		796		205	
3	4,500,000	15		15		15	0	15	0	15	0	18	0		0		1			
4 C	4,500,000	15		15		15	0	15	0	15	0	15	2		120		366		2130	
5 c	4,500,000						0		0		1		5		43		336		65	
6	6,000,000	25		15		12	0	17	0	16	1	24	2		1		3		34	
7 c	6,000,000						0		0		10		111		784		952		516	
8	500,000,000			25			0	5	1	5	6	5	4	5	11	5	14		36	
9 c	500,000,000						6		32		226		400		1440		5732		D	
10	1,000,000			5		5	0	5	0	5	0	5	0		0		5		0	
11	1,000,000			10		10	0	10	0	10	0	10	0		0		0		8	
12 c	1,000,000						0		0		0		0		0		0			
13	3,370,000			5		5	0	5	0	5	5	5	24	5	33	5	836	5	66	
14	3,370,000			5		5	0	5	1	5	5	5	6	5	136	5	278	5	383	
15	3,370,000			5		5	0	5	1	5	1	5	4	5	47	5	26		150	
16	3,370,000			5		5	0	5	1	5	2	5	4	5	37	5	60		92	
17	3,370,000						0		0		2		4	5	74	5	440	5	769	
18	3,370,000						0		0		1		5	5	5	5	123	5	355	
19 C	3,370,000			5		5	0	5	0	5	1	5	1	5	6	5	18	5	28	
20 C	3,370,000			5		5	1	5	0	5	2	5	75	5	96	5	1605	5	511	
21	1,407,000			5		5	0	5	0	5	0	5	0	5	0	5	1	5	0	
22	1,407,000			5		5	0	5	0	5	0	5	0	5	0	5	2	5	2	
23	1,407,000			5		5	0	5	0	5	0	5	0	5	1	5	9		23	
24	1,407,000			5		5	0	5	0	5	0	5	0	5	1	5	1		2	
25	1,407,000						0		0		7	5	17	5	112	5	612	5	1775	
26 C	1,407,000			5		5	0	5	0	5	0	5	25	5	115	5	654	5	1483	
27 C	1,407,000			5		5	0	5	0	5	4	5	10	5	84	5	250	5	1321	

D = died. S = survived. C = control, received normal monkey serum. c = control, receive

LE I

Various Intervals Following Inoculation with the Malaria Parasites (Plasmodium knowlesi) and also the ing Blood of the Infected Animals

llowing inoculation with parasites

8		9		10		11		12		13		14		15		16		17		18		19		
Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	Amount serum	Parasite count	
cc.		cc.		cc.		cc.		cc.		cc.		cc.		cc.		cc.		cc.		cc.		cc.		
	81		203		1300		1160		2840		D													
	6186		D																					
	24		6		32		10		162		51		49		84		51		148		12		7	S
	6840		D																					
	1460		3354		1648		3156		D															
	65		63		308		360		750		349		154		25		7		2		—		195	S
	D																							
	331		844		651		1174		1848		962		1767		6039		D							
	2		46		190		184	5	470	5	1098	3	72		218		140		370		165		96	S
	13		73		89		368	10	1152	10	504	5	145		80		48		159		53		91	S
	1		1		7		93	10	125	10	299		778		804		887		2985		4716		1710	D
5	2645	5	5215		D																			
5	1166	5	932	5	4036		D																	
	210		396		435		333		152		97		68		64		69		16		6		0	S
	32		32		41		41		103		32		95		32		29		78		95		6	S
5	290	5	139	5	100	5	93		32		2		1		14		76		34		52		28	S
5	366	5	804	5	1449		D																	
5	120	5	242	5	467	5	932	5	2056		524	5	3780		5130		D							
	D																							
5	12	5	20	5	174	5	235	5	172	5	756		1402		D									
5	11	5	6	5	74	5	97	5	366	5	740		2805		6552		D							
	45	10	637	10	1802	10	3702		D															
	23	10	103	10	308	10	2542	10	1968	10	3575		D											
	6812		D																					
	D																							
	D																							

d no serum.

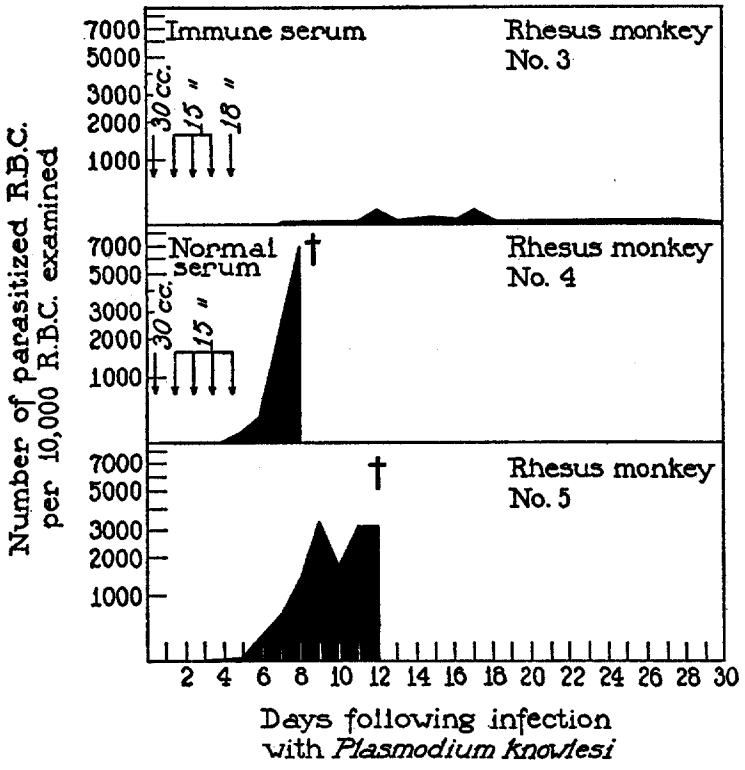

CHART 2

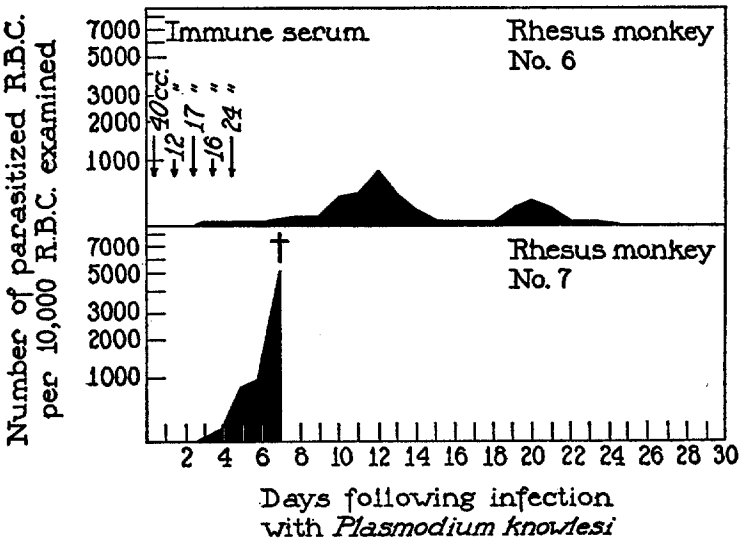

CHART 3

passive immunity to normal animals, it was decided to subject the protective property of the serum to a more severe test.

Experiment 4.—Two normal monkeys, Nos. 8 and 9, were each inoculated with 500,000,000 parasites, which was approximately 100 times the number used in the previous experiments. Monkey 8 had received 25 cc. of immune serum 3 hours prior to the injection of the parasites and 5 cc. daily for 5 days thereafter. The results are shown in Chart 4 and Table I. It will be noted that in spite of inoculation with an overwhelmingly large number of parasites and the use of a relatively small amount of immune serum, monkey 8 had a prolonged course of infection and

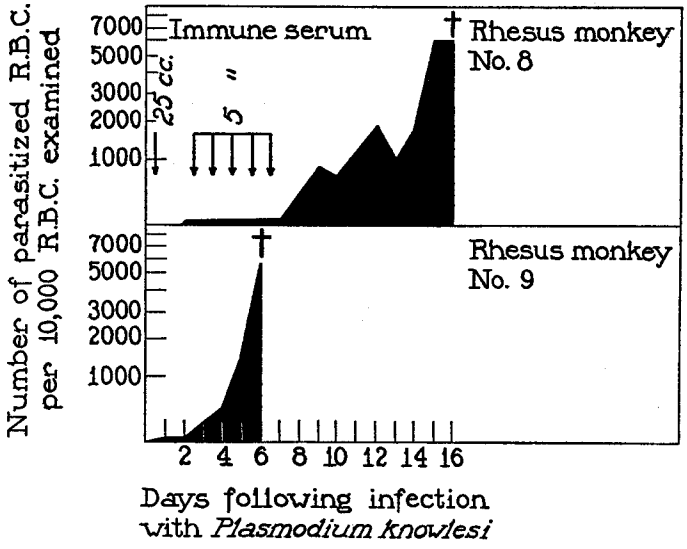

CHART 4

did not die until the 16th day of the disease, whereas the control animal, No. 9, died on the 6th day after inoculation.

An experiment was now undertaken to determine the protective property of varying amounts of immune serum against a relatively small infecting dose of the parasites.

Experiment 5.—Three normal monkeys, Nos. 10, 11, and 12, were each inoculated with approximately 1,000,000 parasites. Monkey 10 received 5 cc. of immune serum daily for 5 days, and monkey 11 was given daily injections of 10 cc. for a similar period, while monkey 12 served as control. It is frequently observed that when the infecting dose of the parasites is small, the incubation period is

considerably prolonged. In this experiment the control monkey, No. 12, did not die until the 19th day after inoculation. After a prolonged incubation period monkeys 10 and 11 showed an intensity of infection similar to the control animal. However, when additional injections of immune serum were given at the periods as indicated in Chart 5 and Table I, there was a rapid fall in the number of parasites present in the circulating blood and both animals recovered.

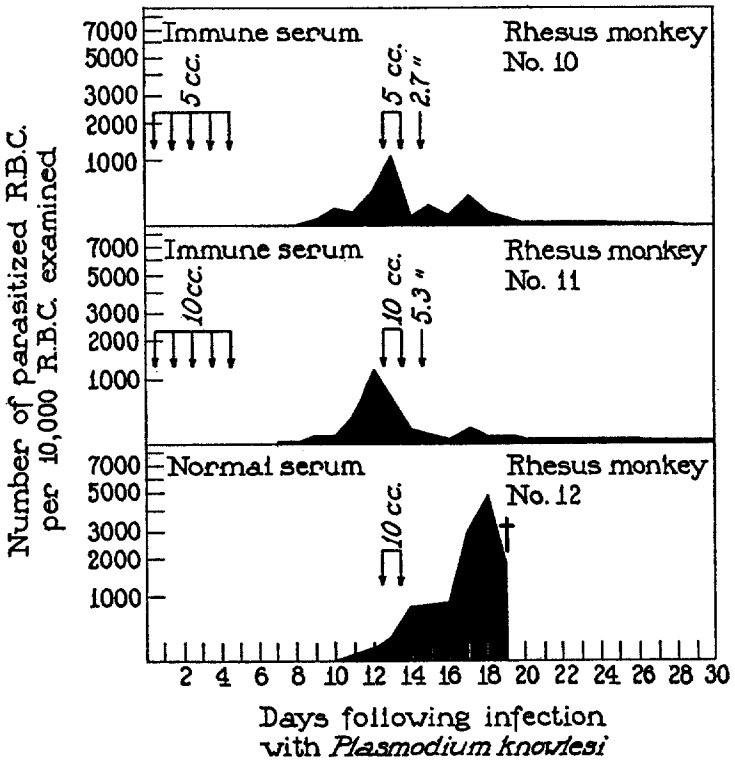

CHART 5

With a view to determining the most favorable time for the administration of immune serum in order to afford maximum protection, the following experiment was carried out.

Experiment 6.—Eight normal monkeys, Nos. 13 to 20 inclusive, were each inoculated with 3,370,000 parasites. As shown in Table I, monkeys 13 and 14 were given 5 cc. of immune serum daily during the entire course of the disease;

Nos. 15 and 16 each received 5 cc. daily during the first 7 days; Nos. 17 and 18 were given no serum until the infection had become well established, which was

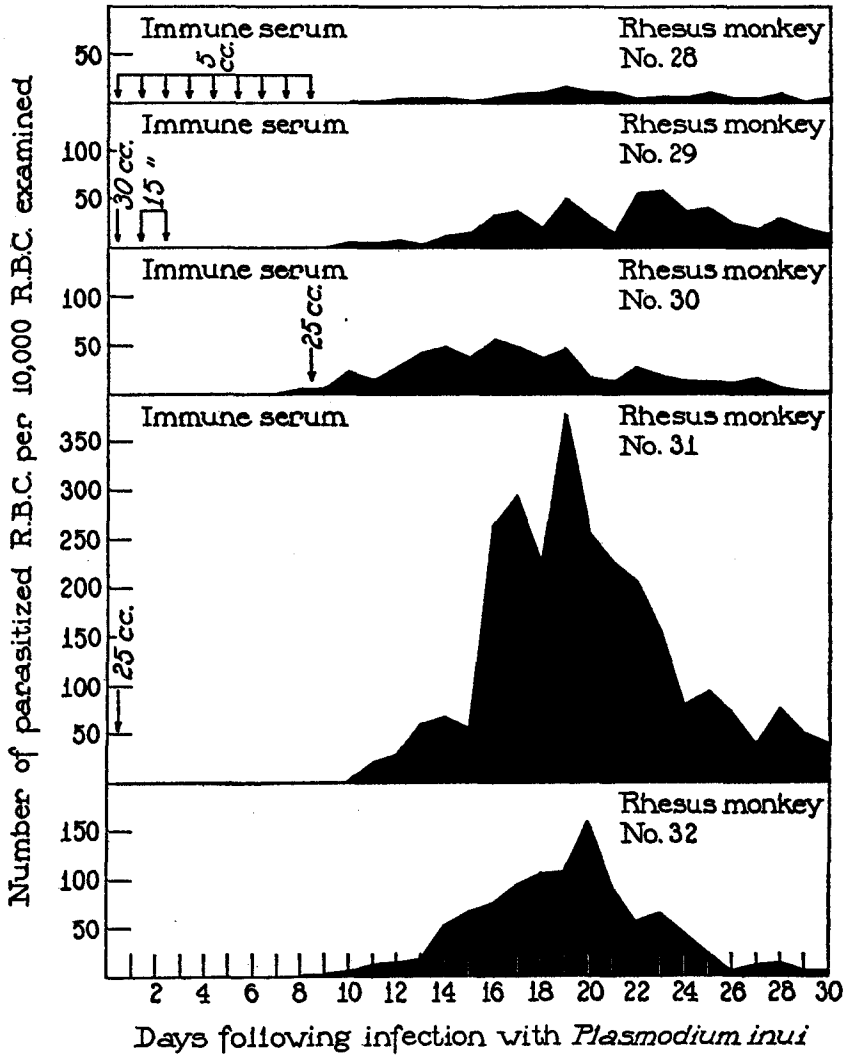

CHART 6

on the 5th day, and then were given 5 cc. daily for the duration of the disease. The control monkeys, Nos. 19 and 20, each received 5 cc. of normal monkey serum

daily throughout the course of their infection. As seen in Table I, monkeys 13 and 14 died of acute malaria on the 10th and 11th day, respectively; Nos. 15, 16, and 17 survived, while No. 18 also died on the 10th day after inoculation. The control animals, Nos. 19 and 20, died on the 16th and 8th day, respectively.

The results seemed to indicate that while the immune serum afforded good protection in some animals, it completely failed in others. We have no adequate explanation for this phenomenon, but it is suggested that besides individual variation in the susceptibility of monkeys, there may be a considerable variability in the concentration of the immune substances in the serum of individual monkeys harboring chronic infection from which the pools were made up.

The preceding experiment was repeated except that a different pool of immune serum was used and the infecting inoculum contained a smaller number of parasites.

Experiment 7.—Seven normal monkeys, Nos. 21 to 27 inclusive, were each inoculated with 1,407,000 parasites and the immune serum was given at the same intervals as in Experiment 6. The results are shown in Table I. Monkeys 21 and 22, which received 5 cc. of serum daily throughout the course of infection, died on the 15th and 16th day, respectively, after injection of the parasites; monkeys 23 and 24, each of which was given 5 cc. of immune serum daily during the first 7 days of infection, died on the 12th and 14th day, respectively. Monkey 25, which received 5 cc. of immune serum daily, commencing after the infection was well established, died on the 8th day. The two control animals, Nos. 26 and 27, both died on the 7th day after inoculation.

It seemed obvious from these results that the immune pool used in this experiment had only a slight protective property, as shown by the prolongation of the course of infection in monkeys which received daily injections of 5 cc. each throughout the disease. This serum appeared to have been ineffective when given after the infection had already become firmly established.

All the foregoing experiments were carried out with *P. knowlesi*. In order to determine whether a chronic infection with another species of malaria parasite results in the production of protective substances in the serum of the host, the experiment described below was carried out with *P. inui*. When injected into *rhesus* monkeys *P. inui* generally produces only a moderately severe infection which practically always is followed by spontaneous recovery. A number of monkeys

which had recovered from an initial infection with this parasite were bled and the sera pooled. The protective effect of this serum against *P. inui* infection was determined by the intensity of infection as shown by daily counts of parasitized cells per 10,000 normal red cells.

Experiment 8.—Five normal monkeys, Nos. 28 to 32 inclusive, were each inoculated with 2,500,000 parasites. The results of this experiment are shown in Chart 6. Monkey 28, which received 5 cc. of serum for 9 consecutive days after the administration of parasites, had a maximum count of 16 parasites per 10,000 normal red blood cells on the 19th day of infection. Monkey 29, which received 15 cc. of serum 2½ hours before inoculation with parasites, 15 cc. at the time of infection, and 15 cc. daily for 2 days, had a maximum count of 59 on the 23rd day of the disease. Monkey 30 received 25 cc. of serum on the 8th day after the onset of infection and none thereafter; the maximum count was 56 on the 16th day. Monkey 31 received only one serum injection of 25 cc. This was administered 2½ hours before inoculation with parasites and the maximum parasite count was 380 on the 19th day. Monkey 32, the control, received no serum and had a maximum count of 160 which occurred on the 20th day of the infection.

It is to be noted in this experiment that serum from animals which had recovered from the acute attack due to *P. inui* was most effective in decreasing the intensity of the infection when administered during the course of the disease.

DISCUSSION

There are numerous reports in the literature which suggest the formation of specific antibodies in the host during the course of an infection due to malaria parasites. In avian malaria, for example, the recovery from an initial attack occurs with such rapidity that it is often referred to as a crisis. Since it is known that from the onset of the infection the macrophages of an infected bird are constantly phagocytizing parasites, the marked decrease in their number at the time of recovery could probably best be explained on the basis of opsonizing antibodies or humoral immune substances. Furthermore, the fact that an animal after recovery from a malarial infection is usually immune to reinfection with the homologous parasite would tend to suggest that there are specific protective substances present in the serum of such an animal. These observations have led Taliaferro and Cannon (7), Ciuca (8, 9), Hackett (10), Ferrio (11), Thomson (12), Neumann (13), and others, to infer that specific humoral immune

substances to malaria parasites are present in the serum of the host during chronic infection. But it is usually pointed out that these antibodies appear in the serum in such a low concentration that their presence cannot be demonstrated by ordinary protection test methods.

We believe that the data presented in this paper offer definite evidence to indicate that, in some instances at least, protective substances do occur in the serum of monkeys harboring chronic infection with *P. knowlesi* and *P. inui*, and that the serum of such animals, when injected into normal monkeys, is capable of conveying passive immunity in the animal recipient to the homologous parasites. Our results also indicate that the protective antibodies appear in the serum of animals with chronic infection in a very low concentration, and that large amounts of the serum must be used in order to demonstrate their presence. In our experiments amounts varying from 22 to 109 cc. were given to an animal, which makes an average of 52 cc. per animal, or 26 cc. per kilo of body weight. Pooled serum from a number of monkeys was used in these experiments, and as shown by the results there was a considerable variation in the protective property of different pools. This would seem to suggest that a wide variation probably exists in the degree of humoral immunity in monkeys suffering from chronic malaria.

Our results suggest that the protective action of the immune serum was most pronounced when the serum was administered in daily doses throughout the course of the experimental disease. Relatively large amounts given shortly before or at the time of the injection of the parasites seemed to have only a minor influence on the course of infection. Likewise, when given after the infection was already well established the serum seemed to have little effect on the final outcome. Of special interest are monkeys 10 and 11, in Experiment 5, which received immune serum both early and late in their infection. As seen in Table I, after the first course of serum treatment had ceased, the parasite count in the blood of these two monkeys rose to 1098 and 1152, respectively. Immune serum given at this point resulted in a fall in the parasite count to 72 and 504, respectively, within 24 hours. It has often been observed that when the parasite count has reached 1000 in an initial attack of *P. knowlesi* infection it is impossible to prevent death even with massive doses of quinine.

In preparing the immune serum pools used in the experiments described above, a considerable number of the monkeys with chronic malarial infection were bled repeatedly and some as many as six times. Red blood cell counts done at frequent intervals on these animals showed that it required about 3 weeks after each bleeding before the normal counts were restored. After bleeding these monkeys frequently showed temporary macrocytosis and polychromasia. Parasite counts usually showed little or no alteration after the multiple bleedings. One animal, however, suffered a relapse and died of acute malaria in spite of massive quinine therapy. Following successive bleedings of the original monkeys with chronic infection, the potency of the pooled immune serum seemed to decrease progressively as evidenced by its ability to protect normal animals.

SUMMARY AND CONCLUSION

A *Plasmodium knowlesi* infection in *rhesus* monkeys is almost invariably fatal. This infection, however, may be made chronic by the early administration of antimalarial drugs. The animals then will harbor a chronic infection for an indefinite period. The serum taken from monkeys with chronic infection and injected into those suffering from an acute attack was found to have a definite depressing effect upon the course of the experimental disease. In some instances death was prevented and the acute infection changed into a chronic form; in others, the course of the experimental disease was prolonged.

In a similar manner the serum from monkeys harboring a chronic *Plasmodium inui* infection, when injected into monkeys suffering from an acute attack due to this parasite, was found to be effective in reducing the intensity of the primary infection.

The data presented indicate that protective antibodies are produced in the serum of monkeys during experimental malaria infection.

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