sites reached 7.6 per 100 red blood cells. Of the control ducks three showed more than 80 parasites per 100 red blood cells and the other 54 parasites per 100 red blood cells. Two of these ducks died. In the second experiment the protection was almost as great as in experiment 1. None of the control ducks which died showed on autopsy any gross changes indicating death from any cause other than malaria.

Discussion

Although we have no experiments with malaria on this point, other experiments[5] in which the adjuvants mentioned above were used combined with various antigens suggest that one single injection of malarial parasites plus adjuvants may be sufficient to produce the results described above and that the protection may last for a very long time. It is also possible that similar results could be obtained without tubercle bacilli or that timothy-grass bacilli can be substituted for tubercle bacilli.[5b]

In the interpretation of our results it may be mentioned that the absence of parasites in 500 red blood cells does not indicate that there are no parasites in the blood or in the organs. Thus in these experiments the actual immunity may be less than the apparent disappearance of the parasites from the blood of the immunized ducks suggests.

Summary

When ducks are injected with formalin-inactivated *P. lophurae* in combination with a lanolin-like substance, paraffin oil and killed tubercle bacilli, they develop considerable resistance to subsequent infection with *P. lophurae*.

Jules Freund
Harriet E. Sommer
Annabel W. Walter

Bureau of Laboratories, Department of Health and the Public Health Research Institute of the City of New York, Inc.

IMMUNIZATION OF RHESUS MONKEYS AGAINST MALARIAL INFECTION (P. KNOWLESI) WITH KILLED PARASITES AND ADJUVANTS[1,2]

Eaton and Coggeshall[3] reported that numerous injections of large doses of *P. knowlesi* parasites killed by formalin or other means do not produce sufficient immunity in Rhesus monkeys to protect them against lethal infection with the same strain of parasite. Coggeshall[4] found, however, that immunity did develop in monkeys with chronic infections (carriers) since they were resistant to newly introduced parasites (*P. knowlesi*) of the same strain. Moreover, Coggeshall and Kumm[5] have shown that serum from monkeys harboring a chronic infection with *P. knowlesi* contains specific, protective antibodies since such serum hinders the progress of the disease in susceptible monkeys.

In this study Rhesus monkeys were immunized with formalin-killed *P. knowlesi*[6] parasites combined with a lanolin-like substance and paraffin oil containing killed and dried tubercle bacilli, for these substances proved to be effective in enhancing and sustaining antibody production with many antigens.[7] The antigen was prepared as follows: Citrated monkey blood containing large numbers of parasites were centrifugalized immediately after collection. The sediment was washed in 0.85 per cent. saline solution and suspended in 0.85 per cent. saline solution containing 0.1 per cent. formaldehyde. The suspension was kept in the refrigerator over night and washed three times with 0.85 per cent. saline solution. The sediment after the third washing was used for the unconcentrated vaccine. Concentrated vaccine was prepared by suspending the sediment of the third washing of formalinized red blood cells in 0.85 per cent. saline solution. After 48 hours in the cold the suspension was centrifugalized lightly and the reddish-brown cell-stroma-parasite layer was collected. The centrifugalization and the collection of parasitized cells was repeated several times. The final suspension contained large numbers of parasites and ghosts of red cells and very few normal red blood cells, whereas the residual red blood cell layer contained few parasites.

A water-in-oil suspension of the antigen in paraffin oil containing killed and dried tubercle bacilli was made with the aid of "Falba,"[8] the proportion of antigen, oil and "Falba" being 2:2:1 (Table 1).

[1] Manuscript completed September, 1944. See preceding paper.

[2] This study was aided by a grant from the John and Mary R. Markle Foundation.

[3] M. D. Eaton and L. T. Coggeshall, *Jour. Exp. Med.*, 70: 141–146, 1939.

[4] L. T. Coggeshall, *Medicine*, 22: 87–102, 1943.

[5] L. T. Coggeshall and H. W. Kumm, *Jour. Exp. Med.*, 68: 17–27, 1938.

[6] The strain of *P. knowlesi* was received through the courtesy of Dr. Johannes H. Bauer, of the International Health Division of the Rockefeller Foundation, to whom we are indebted for helpful advice and suggestions.

[7] (a) J. Freund and K. McDermott, *Proc. Soc. Exp. Biol. and Med.*, 49: 548–553, 1942; (b) K. Landsteiner and M. W. Chase, *Proc. Soc. Exp. Biol. and Med.*, 49: 688–690, 1942; (c) M. W. Chase, *Proc. Soc. Exp. Biol. and Med.*, 52: 238–240, 1943; (d) L. M. Kopeloff, S. E. Barrera and N. Kopeloff, *Am. Jour. Psychol.*, 98: 881–902, 1942, L. M. Kopeloff and N. Kopeloff, *Jour. Immun.*, 48: 297–304, 1944; (e) E. A. Kabat and M. H. Boldt, *Jour. Immun.*, 48: 181–183, 1944; (f) J. Freund and M. V. Bonanto, *Jour. Immun.*, 48: 325–334, 1944; (g) W. F. Friedewald, Science, 99: 453–454, 1944; (h) J. Freund and A. W. Walter, *Proc. Soc. Exp. Biol. and Med.*, 56: 47–50, 1944.

[8] Falba is said to be a mixture of oxycholesterins and cholesterins derived from lanolin (manufactured by Pfaltz and Bauer, Inc., New York, N. Y.).

TABLE 1

COMPOSITION OF VACCINE AND SCHEDULE OF INJECTIONS

Experiment	Monkey	Materials injected per monkey							Days between	
		Dose	Volume cc	Parasites billion	Red-cells billion	"Falba" cc	Paraffin oil cc	Killed dried tubercle bacilli mgm	Sites injected	Vaccinations	1st dose and challenge
1	15*	1st	2.26	4.5	15.4	.45	0.9	.45	3		
		2nd	7.5	3.0	12.6	1.5	3	1.5	3	46	
		3rd	7.5	14.7	25.9	1.5	3	3.0	3	28	118
	16*	1st	12.5	5	20.8	2.5	5	2.5	5		
	17*	2nd	7.5	14.7	25.9	1.5	3	3.0	3	28	71
	18*	1st	7.5	14.7	25.9	1.5	3	3.0	5		
		2nd	15.0	15	45.6	3	6	2.5	5	56	92
2	19†	1st	2.5	16‡	?§	0.5	1	2.5	5		
	20†	2nd	5.5	9.9‡	?§	1.1	2.2	1.4	5	52	86
	21†										

* Unconcentrated vaccine.
† Concentrated vaccine.
‡ Estimated from the red cell and parasite counts made on the unconcentrated blood.
§ Unknown.

Details of the procedure for making water-in-oil emulsions may be found in a previous paper.[7]

Each dose of vaccine was divided into three or five equal portions and injected into the subcutaneous tissue of the axillae, back of neck and groins. Subsequently monkeys so vaccinated (Table 1) and unvaccinated monkeys were infected by the intravenous injection of 2 to 3 cc of heparinized blood from an infected monkey which had been made a carrier by treatment with quinine.

Two experiments were carried out. In Experiment 1, there were 2 controls and 3 immunized monkeys; in Experiment 2, 6 controls and 4 immunized monkeys.

The parasite counts are shown in Tables 2 and 3. Table 2 contains the data on the unvaccinated monkeys of Experiments 1 and 2. It also includes 6 monkeys which received intravenous injections of 2 to 3 cc of blood from the carrier monkey, though

TABLE 2

PARASITEMIA IN NON-VACCINATED MONKEYS
PARASITES PER 100 RED BLOOD CELLS

Days after infection	Monkey number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	–	–	–	0	–	0	0	–	–	–	–	–	–
2	0	–	–	–	–	–	0	0	0	0	0	0	0	0
3	0	–	0	.4	0	–	0	0	–	–	–	–	–	–
4	0	–	.3	.3	0	–	0	0	0	0	0	0	0	0
5	0	0	–	–	–	0	.1	÷	0	0	0	0	0	0
6	0	0	14	10	.8	0	÷	.3	0	0	0	.1	0	0
7	0	÷	57K	53K	2	0	2	6	÷	0	0	÷	÷	.1
8	0	÷			9	0	40K	37K	1	.5	.1	.1	2	.5
9	0	1			D	0			10	.7	.7	8	8	3
10	÷	7				0			4	3	6	25K	24K	6
11	2.4	10				0			9K	9K	D			8
12	10.2	24K				0								5
13	36.0D					1.5								6
14						D								4
15														2
16														.3
17														0
18														.9
19														1
20														.4
21														0
22														0
23														0
24														–
25														0
26														0

– Not done.
÷ One parasite in more than 1000 r.b.c.
K = Killed.
D = Dead.
Experiment 1: Monkey Nos. 7, 8.
Experiment 2: Monkey Nos. 9, 10, 11, 12, 13, 14.
Monkey Nos. 1 through 6 were infected with the blood of the carrier but not simultaneously with animals in Experiments 1 and 2.
Autopsy: No tuberculosis: Monkey Nos. 1, 2, 3, 4, 7, 9, 11, 12.
Tuberculosis: Monkey Nos. 5, 6, 8, 10, 13.

they were not infected on the same days as the monkeys in Experiments 1 and 2. These animals may also be considered controls since Coggeshall and Eaton[8] demonstrated that the number of parasites introduced influences only the length of the prepatent period. Once the parasites become demonstrable, the progress of the disease is almost uniform, with death occurring usually on the 3rd to 6th day of the parasitemia. Table 2 shows that in the unimmunized animals parasites were first found in thin blood smears from 3 to 8 days after infection, and thereafter multiplied rapidly. Four of the 14 monkeys died with acute malaria. Nine monkeys were killed (to recover parasites for vaccine production). One unimmunized monkey survived after an infection which at its peak showed 8 parasites per 100 r.b.c. Mulligan and Sinton observed that 1 of 120, and Coggeshall and Kumm[5] 1 of 70 monkeys recovered spontaneously from an infection with *P. knowlesi*.

TABLE 3

PARASITEMIA IN VACCINATED MONKEYS
PARASITES PER 100 RED BLOOD CELLS

Days after infection	Unconcentrated vaccine			Monkey number	Concentrated vaccine		
	15	16	17	18	19	20	21
1	0	0	0	–	–	–	–
2	0	0	0	0	0	0	0
3	0	0	0	–	–	–	–
4	0	0	0	0	0	0	0
5	0	0	.1	0	0	0	0
6	÷	0	.1	0	0	0	0
7	1	0	.2	0	0	0	0
8	10	0	3D	0	0	0	0
9	4	0		0	0	0	0
10	8	.1		0	0	0	0
11	5	0		.1	0	.1	.1
12	6	.6		.4	0	.4	0
13	2	2		.5	0	1	.2
14	.7	2		.5	0	.6	1
15	.3	1		2	0	1	.5
16	.1	.4		2	0	.6	.6
17	.2	0		.5	0	.3	.1
18	.1	.1		2	0	.4	0
19	.2	0		6	0	.7	0
20	0	0		3	0	.3	0
21	.1	0		.2	0	.2	0
22	0	0		0	0	0	.1
23	.2	0		0	0	0	0
24	÷	.1		–	–	–	–
25	0	0		0	0	0	0
26	.1	0		0	0	0	0

Experiment 1: Monkey Nos. 15, 16, 17.
Experiment 2: Monkey Nos. 18, 19, 20, 21.
Monkey No. 15 died with extensive tuberculosis on the 69th day after infection. From the 27th to the 68th day it was examined for parasitemia on 21 different days. Parasites were found on 9 days, being 1 in 1,000 or more r.b.c. On the 68th day, one parasite was found in more than 1,000 r.b.c.
Monkey No. 16 died of tuberculosis on the 49th day after infection. From the 27th to the 49th day it was examined for parasitemia on 15 different days. No parasites were found.
Monkey No. 17 died in acute respiratory distress while a blood smear was being taken. Nine tenths of the cut surface of the lungs were involved in tuberculosis.

Table 3 shows the parasite counts in immunized animals. In one of 7 monkeys parasites were not found in thin blood smears. In the other six animals there were only 1, 1, 2, 3, 6 and 10 parasites per 100 r.b.c. at the peak of infection. Subsequently the parasites decreased until none were demonstrable in thin smears.

Although the number of monkeys in the experiments was small the difference between the course of infection in vaccinated and non-vaccinated monkeys seems significant considering the high virulence of *P. knowlesi* for the Rhesus monkey.

Palpable masses were found in the subcutaneous tissue at the sites of injection in all vaccinated monkeys. These masses did not ulcerate through the skin in the three monkeys which showed no reaction to tuberculin (P.P.D.). However, it may be noted that one of these monkeys (No. 15) showed extensive tuberculosis at autopsy 85 days after its negative reaction to P.P.D. Abscess formation and ulceration through the skin were observed in the four monkeys which reacted to P.P.D. Two of these animals died and showed extensive tuberculosis at autopsy.

CONCLUSION

The injection of formalin killed *P. knowlesi* parasites combined with a lanolin-like substance and paraffin oil containing killed tubercle bacilli modifies parasitemia and prevents fatal infection with *P. knowlesi* in Rhesus monkeys.

JULES FREUND
K. J. THOMSON
HARRIET E. SOMMER
ANNABEL W. WALTER
EDNA L. SCHENKEIN

PUBLIC HEALTH RESEARCH INSTITUTE
OF THE CITY OF NEW YORK, INC.
AND THE DEPARTMENT OF HEALTH,
BUREAU OF LABORATORIES,
CITY OF NEW YORK

SCIENTIFIC APPARATUS AND LABORATORY METHODS

METHOD FOR THE DETECTION OF INDOLE[1]

THE detection of indole is a matter of importance in a number of different fields. For instance, principal uses of this test are (1) the differentiation of bacteria, some of which characteristically produce indole while others do not[2]; (2) the detection of spoilage of foods[3]; (3) the demonstration of the

[1] Contribution from Department of Health, City of New York.

[2] Am. Pub. Health Assoc. Standard Methods of Water Analysis. 8th ed. 1936.

[3] Clarke, Cannon, Coulter, Goodman, Greene, Milsted, Vandaveer and Wildman, *Jour. Assoc. Official Agr. Chem.*, 20: 475, 1937; *Chernoff, Ind. Eng. Chem.*, Anal. Ed., 12: 273, 1940.