tion. Actually, the degree of prolongation may be greater as the assay methods used by the above investigators gave values as low as .01 unit per cc, whereas the method in this report had a minimum reading of .1 unit per cc.

TABLE 2

SHOWING DEGREE TO WHICH PENICILLIN IS CONCENTRATED BY KIDNEY

Case	Interval Hours	Blood units/cc	Urine units/cc	Concentration
No. 15	2	.225	135	?
	4	.225	135	600
	6		135	600
No. 17	2	.45	90	
	4	.225	90	200
	6	.225	90	400
	8		37.5	150
No. 20	2	.225	90	
	4	.225	135	600
	6	.225	45	200
	8		37.5	150

During this work it became apparent that penicillin was consistently present in the urine in much greater concentration than in the blood stream and that it may be detected much longer. The rate of excretion dropped rather rapidly in the first 6 to 8 hours and then remained fairly constant for 8 to 16 hours despite the low or absent blood levels. The unit excretion per interval of time was for the most part independent of the urinary volume.

It has been suggested that penicillin is excreted from the tubules of the kidney in addition to filtration through the glomeruli. Concentration values were calculated on the basis of urinary concentration in units per cc divided by the blood level at the end of the previous two-hour period. Sample concentrations appear in Table 2 and varied from 100 to 600 times. As the kidney excretes non-threshold substances creatinine and sulfates in concentrations of 75 to 90 times, respectively, the markedly high values found with penicillin can be explained only by renal tubular excretion. Dawson *et al.*[8,9] have shown that blood serum and whole blood do not inhibit penicillin, therefore the persistence of high concentrations of penicillin in urine long after demonstrable blood levels are absent can be explained by the remarkable power of the kidney to concentrate and excrete it. Another explanation might be storage in the tissues, which would tend to make the blood levels lower and prolong the excretion in the urine.

The total amount of penicillin excreted was relatively constant around 68,000 units, which represents 34 per cent. of the original dose given. This rather low total excretion when compared to values of 50 to 60 per cent. excretion after injection of penicillin in water or saline can be explained by its destruction in the body due to its heat lability or to some other factor in which the time element plays a part. Rammelkamp and Keefer[7] have shown that while the liver excretes penicillin in bile, the total excretion is probably small.

The prolonged presence of penicillin in the urine, however, does suggest the possibility of penicillin being clinically available in the body long after our present concepts have led us to believe. It is true that the levels are minute, but for sensitive organisms and in the presence of leucocytes and antibodies they may prove sufficient.

CONCLUSIONS

(1) Penicillin in combination with peanut oil and beeswax is detectable in the blood stream for longer periods of time following its intramuscular injection than when a water or saline suspension is used.

(2) Penicillin is present in the urine in greater concentration and for much longer intervals than in the blood stream, the concentration being 100 to 600 times. This may prove to be of clinical significance and of value in studying renal physiology.[10]

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IMMUNIZATION AGAINST MALARIA: VACCINATION OF DUCKS WITH KILLED PARASITES INCORPORATED WITH ADJUVANTS[1,2]

IN his recent review of immunity in malaria, Coggeshall[3] came to the conclusion that "the acquisition of immunity following the inoculation of killed malarial organisms is only demonstrable under exceptional conditions." Jacobs[4] published evidence indicating that partial immunity against *P. lophurae* in the duck may be obtained by injecting killed parasites in combination with staphylococcus toxoid. In his experiments the immunized ducks were challenged three days after the fifth injection of vaccine and

[5] Herrel, Nichol, Heilman, *Jour. Am. Med. Asn.*, 125: 15, August 12, 1944.
[6] Cooke and Golding, *Jour. Am. Med. Asn.*, 127: 80, January 13, 1945.
[7] Rammelkamp and Keefer, *Jour. Clin. Invest.*, 22: 425, May, 1943.
[8] Rammelkamp and Bradley, *Proc. Soc. Exp. Biol. and Med.*, 53: 30, May, 1943.
[9] Dawson, Hobby, Meyer and Chaffee, *An. Int. Med.*, November, 1943.

[10] It is desired to acknowledge appreciation to Sergeant Hugh Woosley, who performed the laboratory work herein reported.
[1] Manuscript completed December, 1943.
[2] This study was aided by a grant from the John and Mary R. Markle Foundation.
[3] L. T. Coggeshall, *Medicine*, 22: 87–102, 1943.
[4] H. R. Jacobs, *Am. Jour. Trop. Med.*, 23: 597–606, 1943.

some of the animals showed very little if any protection. Recent experiments,[5,6,7] have shown that antibody production against horse serum, diphtheria toxoid, bact. typhosum and other antigens can be enhanced and sustained for a remarkably long time by combining antigens with a lanolin-like substance and paraffin oil, with or without killed tubercle bacilli.

In the present work the same adjuvants were applied to immunization against malarial infection with *P. lophurae* in the two-months-old White Pekin duck. Heavily parasitized duck red blood cells (approximately 100 parasites per 100 red blood cells) were suspended in salt solution containing 0.1 per cent. formaldehyde, kept at 4° C over night and washed three times with saline. A heavy suspension was mixed with a lanolin-like substance (Falba[8]) and paraffin oil with or without killed tubercle bacilli (Table 1). The proportion was one part red blood cell suspension, one part Falba, and one and one half parts paraffin oil. The vaccine was injected in divided doses into the subcutaneous tissue and muscles over the lateral parts of chest wall. The intervals between injections were approximately one month. Formalin-treated parasitized red blood cells produced no infection when injected intravenously in young ducks. Normal ducks of the same age and kept under the same conditions as the immunized ducks served as controls.

TABLE 1

DOSAGE OF KILLED P. LOPHURAE AND KILLED AND DRIED TUBERCLE BACILLI

Injection No.	Experiment 1		Experiment 2	
	Parasites billions	Tubercle bacilli mg	Parasites billions	Tubercle bacilli mg
1	5	1.0	15	1.7
2	21	0.2	12	0.1
3	11	0	11	0

TABLE 2

NUMBER OF PARASITES PER 100 RED BLOOD CELLS

Group	Duck No.	Day after infection 1	2	3	4	5	6	7	8	9	10	11	12
Experiment 1													
Immunized	27	.2	.6	.8	1	5	8	Dead	(1)				
	34	0	.4	.6	.6	.2	.2	0	0	0	0	0	0(2)
	28	0	.6	.2	0	0	0	0	0	0	0	0	0(2)
	36	0	.4	0	.2	.2	0	0	0	0	0	0	0(2)
Control	35	.2	.2	1	3	13	30	63	83	157	Dead		
	31	.4	0	2	3	9	10	24	58	93	77	Dead	
	37	0	.6	.4	1	6	4	16	42	80	83	49	8(3)
	38	0	.2	1	2	3	3	10	30	30	54	28	6(4)
Experiment 2													
Immunized	43	.2	.2	2	4		0	.4	0	0	0	0	0(6)
	41	.4	1	3	–	1		.6	.4	0	0	–	0(5)
	40	.2	0	0	0	0	0	0	0	0	0	0	0(6)
	39	0	0	0	0	0	0	0	0	0	0	0	0(6)
Control	44	.4	.2	1	5	8	21	59	102	128	98	Dead	
	47	.2	0	.8	4	4	21	34	76	88	108	Dead	
	48	0	.4	1	.8	2	2	2	5	16	22	37	22(7)
	45	0	0	.8	1	.2	.6	.2	1	5	7	15	6(8)

– Not done.
(1) 350 ml fluid in the peritoneal cavity.
(2) No parasites found through 23rd day.
(3) Became 0 on 18th day.
(4) Became 0 on 13th day.
(5) Duck 41 received only the first and second injections of vaccine and was infected 4 weeks later. No parasites found through 41st day.
(6) No parasites found through 13th day.
(7) Became 0 on 20th day.
(8) Became 0 on 20th day.

Both immunized and control animals received approximately one billion parasites by intravenous injection about one month after the last immunizing injection. All ducks were examined for parasitemia by counting parasites in at least 500 red blood cells daily for thirty days after the infecting dose.

Two experiments were carried out using four immunized and four control ducks in each. In the first experiment, as Table 2 shows, three immunized ducks had parasites for only a few days and the counts were less than one parasite per 100 red blood cells. The fourth duck (No. 27) died with 350 ml turbid fluid in the peritoneal cavity and a layer of fibrin covering the liver. In this duck the number of para-

[5] (a) J. Freund and K. McDermott, *Proc. Soc. Exp. Biol. and Med.*, 49: 548–553, 1942. (b) J. Freund and M. V. Bonanto, *Jour. Immunol.*, 48: 325–334, 1944.

[6] (a) K. Landsteiner and M. W. Chase, *Proc. Soc. Exp. Biol. and Med.*, 49: 688–690, 1942. (b) M. W. Chase, *Proc. Soc. Exp. Biol. and Med.*, 52: 238–240, 1943.

[7] (a) L. M. Kopeloff, S. E. Barrera and N. Kopeloff, *Am. Jour. Psychiat.*, 98: 881–902, 1942. (b) L. M. Kopeloff and N. Kopeloff, Federation Proceedings (Am. Soc. for Exp. Biol.), 2, No. 1, 99, 1943.

[8] Manufactured by Pfaltz and Bauer, Inc., New York City.

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*.

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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.).