FACTORS IN PLASMA CONCERNED IN NATURAL RESISTANCE TO AN AVIAN MALARIA PARASITE (PLASMODIUM LOPHURAE)

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Natural resistance to the avian malaria parasite, *Plasmodium lophurae*, has been observed to vary with the species, the age, and the physiological state of the host. Duck and chick embryos and young ducklings and chicks uniformly develop severe infections after the inoculation of the erythrocytic stages of this parasite (1–3). The parasites multiply rapidly in these hosts and reach, within a week or less after inoculation, a peak parasitemia of about 800 to 1200 parasites per 1000 erythrocytes. Adult chickens are relatively resistant (1, 4–6). Some strains of the parasite produce in them slight or no infection even after the intravenous inoculation of a number of parasites large enough to give an initial parasite count of 10 to 20 per 1000 red blood cells. Other more virulent strains, inoculated at a similar high dosage, produce moderately heavy infections in some adult chickens and only light infections in others. Adult ducks have been said to be as susceptible as young ducklings (7) and this is certainly true of some of them. However, female ducks which have recently begun laying eggs or are about to begin, and a small proportion of young adult male ducks, have been found to exhibit a striking relative resistance even to very large doses of the parasite (8). The age resistance of chickens and the individual resistance, perhaps associated with endocrine status, of certain adult ducks may be supposed to depend on a variety of physiological factors. One of these factors must be present in the plasma of resistant individuals, for it has now been possible to demonstrate an antimalarial effect of the plasma of such birds when it is injected into young, uniformly susceptible birds infected with *P. lophurae*.

Methods

White Pekin ducks, Rhode Island Red chickens, and White Leghorn chick embryos served as the experimental animals. The ducks were obtained as day-old ducklings and were reared in cages indoors until they were 3 months old. They received a chick-growing mash and ample water. Between the ages of 6 weeks and 3 months they were occasionally bled for purposes not related to the present experiments. When they were 3 months old they were placed in an outdoor pen and were fed a mixture of 50 per cent ground whole wheat, 25 per cent meat scrap, and 25 per cent ground yellow corn, supplemented twice a week with fresh kale, grass, or alfalfa. Ducks to be used for an experiment were again placed in individual indoor cages. All chicks which were used when 1 to several weeks old were likewise obtained as day-old

birds and reared on the chick-growing mash. Adult hens of known age were obtained from the flock maintained at the Rockefeller Institute at Princeton.

The strain (12A) of *P. lophurae* was maintained as 3 substrains: one in ducklings, one in chicks (derived from the duck strain), and one in chick embryos (3) (derived from the chick strain). Each strain was used for experiments with the corresponding type of host. The strain in ducks was subinoculated once a week, using ducklings 2 to 3 weeks old. The strain in chicks was passed every 5 days, using birds 6 to 12 days old. In all cases, passage was effected by the intravenous injection of parasitized blood.

The general scheme of the experiments was a simple one. Young animals (ducklings, chicks, or chick embryos) expected to be of reasonably uniform, high susceptibility were inoculated with *P. lophurae*. They were divided into groups, some of which were treated with plasma from older birds, while others received buffered saline or a saline solution of other materials. Only homologous plasmas were used, so that the experiments were not complicated by reactions to the proteins of a foreign species.

In the chicken experiments, chicks approximately 1 week old and weighing about 70 gm. were inoculated with enough parasitized chick blood (from chicks on the 5th day of their infection) to give a dose of 200 to 400 million parasites per 100 gm. of body weight. All the chicks for a single experiment were inoculated with the same sample of blood, usually pooled from 2 or 3 infected chicks. The inoculations were done sufficiently quickly so that their order had no effect on the ensuing infections. The dosage used gave an initial parasite count of 10 to 20 per 1000 erythrocytes, as determined from a blood film made within a minute after inoculation. In some experiments the chicks were then given their first treatment of plasma or other material (treatment on day 0). In other experiments treatment was not begun until the 2nd day after inoculation. Blood films were prepared daily for 6 days after inoculation. The specimens were always taken before the day's treatment with plasma or saline had been administered. The schedule of treatments varied, but usually consisted of either 4 treatments given on days 0, 1, 2, and 3, or 2 treatments given on days 2 and 3 of the infection. At each treatment each chick received intravenously 3 ml. of heparinized plasma, buffered saline (pH 7.4), or saline solution of other materials. The plasma for each day's treatment was obtained fresh by bleeding 2 or 3 hens, 6 to 12 months old, from the right jugular vein. A solution of 27 mg. of Connaught heparin in 100 ml. of 0.85 per cent sodium chloride solution was used as an anticoagulant at the rate of about 0.7 ml. per 10 ml. of blood to be drawn. The plasmas were pooled. Different hens were used on successive days, so that a single hen was not subjected to more than 2 bleedings in the course of an experiment. Usually the hens continued to lay eggs in spite of the removal of 30 to 35 ml. of blood at each bleeding.

A fraction containing the euglobulins of hen plasma was prepared aseptically by the following method. 100 ml. of fresh pooled heparinized plasma from egg-laying hens was added to 1900 ml. of sterile distilled water. A considerable precipitate formed. The suspension was distributed in 250 ml. centrifuge bottles with rubber caps and was centrifuged for ½ hour at 3000 R.P.M. The nearly clear supernatant fluid was poured off. The yellow sediment was dissolved in enough buffered saline to give a final volume of 33 ml. The solution so obtained had a greenish yellow color and was highly opalescent. Its protein content ranged from 70 to 100 mg. per ml. The solution was used when freshly prepared. Even after only overnight storage in the refrigerator it showed a considerable light yellow precipitate.

In a single experiment with chick embryos, 15 embryos which had been incubated for 14 days were inoculated intravenously with diluted heparinized blood from an infected embryo (3). A blood film was prepared from each embryo immediately after inoculation. Nine

of the embryos were then given intravenously a dose of 0.2 ml. of heparinized plasma from an adult hen. The other 6 embryos were similarly treated with buffered saline. These treatments were repeated on the 2nd day after inoculation. The course of the infection was followed by daily blood films through the 4th day.

In the experiments with ducks it was possible to test the effect of the plasma of an individual adult duck on the course of infection in 5 young ducklings. Usually 5 groups, each consisting of 5 ducklings 6 days old, were injected with enough diluted parasitized duck blood (all from one given donor on the 6th day of its infection) to introduce about 25 million parasites per 100 gm. of body weight. The ducklings weighed approximately 100 gm. at the time of inoculation and were distributed in the groups so as to give a uniform average weight per group. After inoculation the ducklings of one group each received intravenously 5 ml. of buffered saline, while the ducklings of the other 4 groups were similarly treated with the plasmas of 4 different adult ducks. These treatments were repeated on the following day (day 1) and again on the 4th day. Daily blood films were begun on the 2nd day after inoculation and were continued through the 8th day. Ducklings which died were autopsied. On the 10th day the surviving ducklings were killed and autopsied. Each duckling in a group of 5 received 3 treatments with the plasma of the same adult duck. Approximately 45 ml. of blood was taken from each of the adult ducks (using the right jugular vein) at each of the 3 bleedings. Heparin was used as an anticoagulant in the same manner as for the chickens. After the last bleeding, the adult ducks were given a rest period of 4 days, at the end of which time their blood did not show unusual numbers of immature erythrocytes. In most experiments each of the adult ducks was then inoculated with enough parasitized duck blood to give an initial parasite count of 10 to 20 per 1000 erythrocytes. The course of infection in the adult ducks was followed by a blood film made within a minute or two after inoculation and by daily blood films through the 7th or 8th day thereafter. Ducks which survived the infection were killed on the 7th or 8th day and autopsied, as were ducks which died of the infection. Special attention was given to the size and physiological state of the ovary and oviduct and of the testes.

All blood films were stained with Giemsa's stain in the usual manner. In the counts, enough parasites were counted to keep the error within 10 to 15 per cent (9).

RESULTS

Chicks Treated with Hen Plasma or Euglobulin Fraction of Hen Plasma

In all the experiments of this type the chicks which received 2 or more treatments with hen plasma always developed infections with a lower average peak number of parasites than that of comparable untreated chicks or of chicks treated with buffered saline. The day of occurrence of the peak, usually the 5th, was not changed. Some typical results are presented in Tables I and II. In Experiment A (Table I) 2 control groups were included, one which received buffered saline and one which received a 6 per cent solution of bovine plasma fraction V (albumin) in buffered saline. Each of these groups had about twice as high an average peak parasite number as did either the group which received fresh hen plasma or the group which received hen plasma previously kept at 56°C. for ½ hour. Although in this experiment treatment at 56°C. seemed to have slightly impaired the activity of the plasma, such an effect was not noted in other similar experiments.

TABLE I

The Effect of Hen Plasma on the Extent of Parasitemia in Young Chicks Infected with P. lophurae

The infecting dose of parasitized blood was adjusted on the basis of weight and was large enough to give an initial parasite count of about 10 parasites per 1000 red blood cells. All treatments were given intravenously.

Experiment	No. chicks per group	Age of chicks at start	Treatment	Average peak No. of parasites (per 1000 red cells)
		days		
A	5	10	3 ml. 6 per cent bovine plasma fraction V (albumin) in buffered saline on days 0, 1, 2, 3	1020
			3 ml. hen plasma on days 0, 1, 2, 3	470
			3 ml. hen plasma heated ½ hr. at 56°C. on days 0, 1, 2, 3	650
			3 ml. buffered saline on days 0, 1, 2, 3	1020
B	7	6	3 ml. hen plasma on days 0, 1, 2, 3	370
			3 ml. buffered saline on days 0, 1, 2, 3	960
			3 ml. hen plasma on days 2, 3	430

TABLE II

The Removal of the Activity of Hen Plasma on P. lophurae Infections in Young Chicks by Heating at 65°C.

The chicks were infected when 6 days old. Each group consisted of 7 chicks for Experiment C and 8 chicks for Experiment D. The treatments were given on days 2, 3, and 4 for Experiment C and on days 2 and 3 for Experiment D.

Experiment	Treatment	Average peak No. of parasites (per 1000 red cells)
C	3 ml. hen plasma	860
	3 ml. hen plasma heated ½ hr. at 56°C.	880
	3 ml. buffered saline	1120
	3 ml. hen plasma heated ½ hr. at 65°C. and cleared by centrifuging	940
D	3 ml. hen plasma	627
	3 ml. buffered saline plus heparin at the same concentration as in the plasma	874
	3 ml. hen plasma heated ½ hr. at 65°C. and cleared by centrifuging	909

Experiment B illustrates the fact that 2 treatments (on days 2 and 3) were as effective as 4 treatments (on days 0, 1, 2, and 3) in reducing the average peak number of parasites. However, when the 4 treatments were given a reduction in the average parasite count was apparent on each of the days

following the treatment, whereas this was not the case when 2 treatments were given. This difference is illustrated in Table III and will be discussed again in connection with Experiments E and F. The plasma from roosters was used in a few experiments and seemed to be about as effective as hen plasma.

In most of the experiments the difference between the average peak parasite numbers of the chicks treated with plasma and the control group could be shown to be statistically significant. By the "*t*" test of Fisher (10) the probability (*P*) of the difference being due to chance was 0.02 to 0.01 or less. The critical ratio (the difference of the means divided by the square root of the sum of the squares of their standard errors) was over 2. In 2 experiments

TABLE III

The Effect of a Water-Insoluble Fraction of Hen Plasma on the Course of Infection with P. lophurae in Young Chicks

The chicks were infected when 2 weeks old.

In Experiment E each group consisted of 8 chicks which were treated on days 2 and 3. In Experiment F each group consisted of 10 chicks which were treated on days 0, 1, 2, and 3.

Experiment	Treatment	Average parasites per 1000 red cells on day					
		0	1	2	3	4	Of peak
E	3 ml. buffered saline	13	24	57	209	555	844
	3 ml. plasma fraction in buffered saline (about 80 mg. protein per ml.)	12	29	65	236	482	670
F	3 ml. buffered saline	12	34	74	274	866	915
	3 ml. plasma fraction in buffered saline (about 80 mg. protein per ml.)	12	25	51	172	529	698

the differences were small and not significant statistically. Experiment C (Table II) presents the results of one of these. Note that even in this experiment the average peak parasite numbers for the groups treated with fresh plasma and with plasma heated at 56°C. coincided and were somewhat lower than those for the groups receiving buffered saline and plasma heated at 65°C.

Experiment D (Table II) provides a better illustration of the inactivation of hen plasma by heating at 65°C. for ½ hour and removal of the coagulated proteins by centrifugation. The possibility that the activity of the fresh plasma depends on some specific protein is strengthened by the results of Experiments E and F (Table III). In both of these experiments the average peak parasite number was significantly lower for the chicks receiving the euglobulin fraction of hen plasma than for the controls. In experiment E, in which treatment was not begun until the 2nd day, the chicks destined to receive the

plasma fraction actually had a slightly higher parasitemia on days 1 and 2 than did the controls. This was still the situation on day 3, but on day 4, following the second treatment, the reverse was true. In Experiment F, in which treatment was begun shortly after inoculation of the parasites, the average parasite count of the group receiving the euglobulin was significantly lower than that of the controls on each of the 4 days following treatment. In this experiment one of the chicks treated with euglobulin showed a decrease in its parasite count from 13 per 1000 red cells just after inoculation to 9 one day later and 4 on the 2nd day. The count then rose to a peak of 136 on the 6th day. Such initial decreases accompanying the period of treatment were occasionally observed in other individual chicks receiving hen plasma, but they have never been noted in any of the control chicks or in untreated chicks of comparable age. They have been frequently seen, however, in adult chickens inoculated with *P. lophurae*.

TABLE IV

The Effect of Hen Plasma on the Course of Infection with P. lophurae in Chick Embryos

The embryos were inoculated when they were 14 days old and were treated soon after inoculation (day 0) and again on day 2 with 0.2 ml. of plasma or saline.

Embryos treated with plasma							Embryos treated with buffered saline						
No.	Parasites per 1000 red cells on day					Day of death	No.	Parasites per 1000 red cells on day					Day of death
	0	1	2	3	4			0	1	2	3	4	
1	3	3	24	68	302	6	10	4	13	67	230	736	5
2	6	12	23	84	254	6	11	11	21	105	304	918	5
3	2	5	14	82	130	6	12	7	24	76	292	720	5
4	3	7	23	87	352	5	13	7	12	70	224	796	5
5	8	20	91	324	760	5	14	8	22	95	314	928	5
6	7	32	90	376	848	5	15	3	15	29	102	342	6
7	5	31	65	220	672	5							
8	6	6	29	63	240	6							
9	5	12	49	172	504	5							

Chick Embryos Treated with Hen Plasma

The results of the experiment with 14-day chick embryos are given in detail in Table IV. Six of the 9 embryos which received plasma developed an unusually low infection, whereas only 1 of the 6 embryos which received saline did so. Two of the embryos treated with plasma showed no increase in parasite number between days 0 and 1, and 6 of them showed as low a count on the 3rd day as the saline controls had on the 2nd day. Although the 2 treatments with plasma delayed death slightly, they did not suffice to save any of the embryos.

Ducklings Treated with Adult Duck Plasma

A series of experiments with ducks and ducklings has provided the most striking evidence for a specific correlation between the antimalarial effectiveness of a plasma and the natural resistance to *P. lophurae* of the bird from which the plasma was obtained. In a preliminary experiment, done in the same manner as the experiments with chickens, ducklings infected with *P. lophurae* when they were 2 days old were treated on days 3 and 4 of their infection with pooled heparinized plasma from uninfected 6 to 8-week-old ducklings or with heparinized buffered saline. The average peak parasite number per 1000 erythrocytes was 954 for the 7 ducklings which received plasma and 1040 for the 7 which received saline. Thus the plasma of 6 to 8-week-old ducklings, known to be fully suceptible to *P. lophurae*, had no influence on the course of the infection in very young ducklings.

The effects obtained with the plasmas of adult ducks varied from none at all to a marked suppression of the infection in the treated ducklings. Table V shows in detail the course of infection in 3 groups of ducklings, one treated with buffered saline and 2 with plasma from 2 adult ducks, one of which was later found to be relatively resistant and the other fully susceptible to *P. lophurae*. In this experiment the ducklings which received plasma from the 6½-months-old female duck (No. 9–35) showed only a slight delay in the development of their infections, which reached an average peak parasitemia not significantly lower than that attained in the ducklings which received saline. Of the 5 ducklings which received plasma from the 7-months-old male duck (No. 9–33), 4 developed mild transient infections and even the fifth had a less severe infection than any of the ducklings in the other 2 groups. Such a variation has been the usual result with the effective plasmas. One or 2 of the treated ducklings showed infections approaching in severity those of the controls, while the other 3 or 4 showed very abortive infections. Such abortive infections have not been seen in ducklings treated with buffered saline. Out of 60 ducklings used as controls all but 3 developed infections with a peak parasitemia of about 800 to 1200 parasites per 1000 red cells, and these 3 had peaks of 604, 646, and 157 respectively.

The average of the peak parasite numbers attained in the ducklings of each group has been used as a measure of the severity of infection in the group. Other measures which could have been used and which would give the same relative results are the average ratio of increase of the parasite number between the 4th and 5th days of the infection and the degree of darkening of the livers of the ducklings as determined at autopsy. The average peak parasite number per 1000 erythrocytes was always close to 1000 for groups of ducklings treated with buffered saline. It was similarly high for most groups treated with the plasma of an adult duck which on subsequent inoculation itself developed a severe infection. But the average peak parasite number

was much lower for groups of ducklings treated with plasma from a duck which on subsequent inoculation developed a light or moderate infection. Table VI shows the results of 3 representative experiments of this kind. A remarkable correlation emerged between the average extent of the parasitemia in the ducklings treated with a particular plasma and the severity of the infection which developed in the donor duck when it was later inoculated with

TABLE V

The Effects of 2 Different Adult Duck Plasmas on the Course of Infection with P. lophurae in Ducklings

The ducklings were inoculated when they were 6 days old. They were treated intravenously on days 0, 1, and 4 with 5 ml. of buffered saline or plasma.

Treatment	Duckling No.	Parasites per 1000 red cells on days (after inoculation)							Average peak parasite No. (per 1000 red cells)	Peak parasite No. of donor duck after subsequent inoculation (per 1000 red cells)
		2	3	4	5	6	7	8		
Buffered saline	1	12	34	65	330	542	964	872	1063	
	2	14	35	87	380	796	1195	D*		
	3	9	26	57	346	618	1005	D		
	4	16	38	78	374	656	950	692		
	5	10	41	71	334	710	1200	955		
Plasma from ♂ duck 9-33	6	9	28	37	152	224	416	674	201	181
	7	8	26	38	136	29	3	0		
	8	11	37	41	82	25	11	0		
	9	9	32	40	34	2	2	1		
	10	9	24	35	73	11	1	0		
Plasma from ♀ duck 9-35	11	6	19	24	75	144	429	840	1012	1174
	12	7	22	44	200	380	894	748		
	13	9	36	42	264	566	1110	885		
	14	10	29	56	272	668	1095	954		
	15	10	30	41	212	378	1120	708		

* D signifies death of the animal.

parasites. This is shown in Fig. 1, which includes the results obtained with 34 adult ducks tested by the transfer of plasma to infected ducklings and by subsequent inoculation. If the chart is divided into quadrants, 15 of the points fall in the right upper section, representing ducks with ineffective plasma and with little resistance to the infection; 9 points fall in the left lower section, representing ducks with effective plasma and with a considerable relative resistance to the infection; only 3 points fall in the left upper section and 7 in the right lower section. Only these last represent any appreciable discrep-

ancy, and it is the type of discrepancy which one might well expect to find. The ducks in this group may very well have had some measure of resistance at the time they were being bled for plasma and later became less resistant, perhaps because of the considerable loss of blood they had suffered.

Of the 12 male ducks represented in Fig. 1 only one had an effective plasma and also showed a high degree of resistance to actual infection. One other was

TABLE VI

The Effects of the Plasma of Different Individual Adult Ducks on Infection with P. lophurae in Ducklings, and Their Relation to the Resistance of the Ducks to Direct Inoculation with the Parasite

Each treated group consisted of 5 ducklings inoculated with *P. lophurae* when they were 6 days old and treated with 5 ml. of plasma or buffered saline on days 0, 1, and 4.

Experiment	Treatment	Source of plasma					Average peak parasite No. of treated ducklings (per 1000 red cells)	Peak parasite No. of donor duck after subsequent inoculation (per 1000 red cells)
		Duck No.	Age	Sex	Activity of ovary	Weight of testes		
			mos.			*gm.*		
	Saline						966	
	Plasma	9-25	6.5	♀	++++		431	132
A	"	9-27	7	♀	+		396	856
	"	9-28	7	♀	±		249	662
	"	9-30	6.5	♀	++++		335	41
	Saline						1063	
	Plasma	9-32	7	♀	±		769	870
B	"	9-33	7	♂		90	201	181
	"	9-35	6.5	♀	++++		1012	1174
	"	9-43	5.7	♀	++++		1017	984
	Saline						1040	
	Plasma	9-40	6.5	♀	++		1085	1160
C	"	9-41	6	♀	±		985	810
	"	9-42	6	♀	+		190	724
	"	9-45	5.5	♂		15	1030	990

resistant to the infection but had ineffective plasma and 3 had effective plasmas but no resistance on direct inoculation. Seven would be classed as fully susceptible by both tests. Ten of the 20 female ducks having some degree of activity of the ovary and oviduct had effective plasmas and were relatively resistant to the infection. Four others had effective plasmas but little resistance. The situation in which the plasmas of adult females with some ovarian activity had a distinct antimalarial effect in the treated ducklings and the ducks themselves showed some resistance is exemplified in more detail in

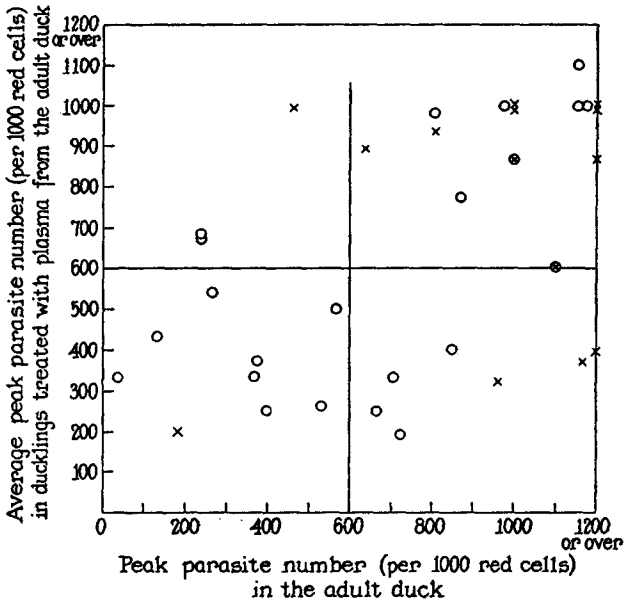

FIG. 1. The correlation between the antimalarial effect of the plasma of individual adult ducks and the severity of their infection after subsequent inoculation.

Each circle represents a female duck with an active ovary, each cross represents a male duck, and each circle with a cross represents a female duck with a completely inactive ovary.

TABLE VII

The Antimalarial Activity of the Plasma of 4 Adult Male Ducks Each Tested Twice at an Interval of 2½ Months

The ducklings were 6 days old when inoculated with *P. lophurae* and each received 5 ml. of plasma or buffered saline on each of days 0, 1, and 4.

Experiment	Treatment	Source of plasma		Average peak parasite No. of treated ducklings (per 1000 red cells)
		Duck No.	Age	
			mos.	
	Saline			847
	Plasma	9-38	7.5	219
A	"	9-39	7	248
	"	9-46	6	1033
	"	9-47	6	208
	Saline			1132
	Plasma	9-38	10	464
B	"	9-39	9.5	445
	"	9-46	8.5	590
	"	9-47	8.5	542

Experiment A of Table VI. Experiment B of this table illustrates the kind of result obtained with several females in very active egg production which had ineffective plasmas and were highly susceptible. Also included in Experiment B was the single male which gave a highly effective plasma and was itself resistant to the parasite. The testes of this male weighed 90 gm. whereas those of the other males weighed 28, 11, 39, 20, 22, 38, 15, 7, 9, 16, and 5 gm. In Experiment C of Table VI one female (No. 9–42) had an effective plasma and was more resistant than another female (No. 9–40) with larger ovary and oviduct. It is interesting that although both ducks 9–40 and 9–41 had laid a few eggs shortly before they were used in the experiment they did not lay eggs thereafter and at autopsy they showed only regressing yolk material with no developing yolks. Duck 9–42 never laid any eggs but its ovary could be seen to be just coming into activity.

In addition to the 12 male ducks represented in Fig. 1, 4 others were tested for the effectiveness of their plasma. These ducks were then not infected but were retested $2\frac{1}{2}$ months later. In the first test, the plasma of 3 of the ducks had a marked inhibitory effect on the infections in the treated ducklings, while that of the 4th had no such effect (Table VII). Two and a half months later all 4 plasmas produced an effect. The smallest was observed with the duck whose plasma had had no effect in the first trial. The plasma of one of the other 3 ducks (No. 9–47) appeared to be less effective than it had been in the first trial. This preliminary experiment indicates a method for following changes with time and physiological status in the resistance of individual animals to infection.

The Influence of Plasma from Resistant Birds on the Reproductive Capability of the Parasite

Taliaferro (11) has pointed out the advantages presented by malaria and other animal parasites for studies of the reproductive capability of an infectious agent (as distinguished from its actual rate of multiplication, which is a resultant of the rate of reproduction and the rate at which the parasites are killed off by various deleterious influences in the host). The reproductive capability of malaria parasites can be measured by determining the number of daughter individuals, or merozoites, produced per segmenter. Counts have been made in several representative experiments of the number of merozoites per 50 completely segmented parasites. When 2 or more segmenters were present in the same host erythrocyte they were not included in the count. In this way it has been possible to compare, at a given point in the course of the infections, the reproductive capability of parasites in young birds receiving saline or a plasma which had no effect on the course of the infection with that of parasites in young birds receiving a plasma which reduced the average severity of the infection. The results are presented in Tables VIII and IX.

It is evident that chicks receiving a euglobulin fraction from hen plasma showed on the 3rd day after inoculation (and hence about 2 days before the peak of the parasitemia) a slightly but significantly lower merozoite number than the control chicks treated with saline. All these chicks had received a total of 3 treatments (on days 0, 1, and 2) at the time the films used for the

TABLE VIII

The Reduction in the Reproductive Capability of P. lophurae Produced by the Treatment of Infected Chicks with a Euglobulin Fraction from Hen Plasma

Counts on the 3rd day after inoculation.

Treatment	Chick No.	Parasites per 1000 red cells	Merozoites per 50 segmented parasites	Average merozoites per 50 segmented parasites	Difference	P*
Buffered saline	1	358	616	629	33	0.02
	2	344	629			
	3	304	642			
	4	238	613			
	5	302	610			
	6	312	616			
	7	140	621			
	8	120	626			
	9	286	630			
	10	340	687			
Euglobulin fraction from hen plasma	11	238	632	596		
	12	146	605			
	13	20	504			
	14	142	615			
	15	150	612			
	16	168	582			
	17	234	580			
	18	198	601			
	19	192	602			
	20	234	628			

* From table of "*t*" (10). A value of 0.02 indicates a just significant difference while values of 0.01 or less indicate a highly significant difference.

counts were made. The average number of merozoites per segmenter was 12.6 for the chicks which received saline and 11.9 for those which received the plasma fraction. Similarly, in 3 separate experiments ducklings that received a plasma which reduced the extent of the parasitemia had segmenters with fewer merozoites than did ducklings treated with an ineffective plasma. The average numbers of merozoites per segmenter were (effective and ineffective plasma respectively): Experiment A 11.3, 12.1; Experiment B 11.6, 12.8; Experiment C 11.6, 12.8.

TABLE IX

The Reduction in the Reproductive Capability of P. lophurae Produced by the Treatment of Infected Ducklings with Plasma from Certain Adult Ducks

Ducklings treated with ineffective plasma served as controls. 5 ducklings were used for each group.

Experiment	Treatment	Day of count after inoculation	Parasites per 1000 red cells	Merozoites per 50 segmented parasites	Average merozoites per 50 segmented parasites	Difference	P*
A	Effective plasma from resistant duck	4	37	553			
			38	571			
			41	556	567		
			40	576			
			35	577			
						40	<0.01
	Ineffective plasma from susceptible duck	4	24	593			
			44	592			
			42	628	607		
			56	610			
			41	613			
B	Effective plasma from resistant duck	5	240	623			
			52	546			
			304	599	579		
			30‡	565‡			
			52	560			
						63	<0.01
	Ineffective plasma from susceptible duck	5	276	668			
			246	626			
			348	643	642		
			360	646			
			278	627			
C	Effective plasma from resistant duck	4	70	579			
			43	574			
			57	583	581		
			48	573			
			47	594			
						60	<0.01
	Ineffective plasma from susceptible duck	4	84	653			
			64	633			
			40	647	641		
			60	631			
			50	642			

* See footnote to Table VIII.

‡ This count was taken on the 4th day, since on the 5th day there were only 2 parasites per 1000 red cells and a sufficient number of fully segmented parasites could not be found.

DISCUSSION

From the experiments described in this paper, the conclusion seems warranted that the relative natural resistance to *Plasmodium lophurae* of adult chickens and of certain adult ducks is at least in part dependent on the presence in their plasma of materials which in some way exert an antiplasmodial effect. The effect is manifest both in a reduced reproduction of the parasites, and in their more rapid and extensive death. It is possible that the similarly decreased merozoite count and the increased mortality of parasites which occur in the later stages of an acute malarial infection (11, 12) may reflect responses to changes in the concentrations in the plasma of antimalarial factors other than the specific antibodies of acquired immunity. It has already been shown that a bound lipid material of the plasma undergoes characteristic changes in concentration during *P. lophurae* or *P. cathemerium* infections in ducklings (13). These changes may be correlated with the stage and the severity of the infection. This material, which is probably a lipoprotein and hence would be precipitated with the euglobulin fraction of plasma, might be the same as or similar to the material responsible for the effectiveness of normal hen plasma and of the plasma of certain adult ducks.

Attempts to demonstrate an effect of hen plasma upon *P. lophurae* cultivated in suspensions of chicken erythrocytes *in vitro* have failed (14). It is difficult to draw conclusions from such a negative result, especially since it was also found that the blood of young ducks recovered from an infection with *P. lophurae* and known to be refractory to superinfection nevertheless supported *in vitro* as good multiplication of the parasite as did blood from a previously uninfected duck of the same age (15).

An antimalarial effect of normal chicken blood, correlated with natural species resistance, has been reported previously by Beckman (16, 17). The sporozoites of *P. cathemerium*, a parasite to which canaries are susceptible but not chickens, survived for 1 to 2 hours when incubated *in vitro* in canary blood or in hen plasma with canary blood cells, but did not do so in hen blood. Non-specific enhancement of the resistance of chickens to *P. gallinaceum* has been obtained by treatment of them before inoculation with normal sheep serum (18) or during the incubation period with a concentrate made from bovine or fowl red cells (19). These results, however, bear no relation to natural resistance to the parasite.

Finally, it is of interest to recall the effects of chicken serum in protecting mice from many lethal doses of pneumococci (20), microorganisms to which chickens are naturally resistant.

SUMMARY

The plasma of adult chickens, when injected into young chicks or chick embryos infected with *Plasmodium lophurae*, lessened the parasitemia. The

substances responsible for this effect were inactivated or removed by the heating of adult chicken plasma for ½ hour at 65°C., followed by centrifugation to remove the coagulated material; but they were not affected by heating for ½ hour at 56°C. The active materials were present in the euglobulin fraction of hen plasma.

In similar experiments with ducks, the plasma from each of a series of adult ducks was tested for its effect on the course of infection in young ducklings. The adult ducks were then inoculated with a large dose of parasites. There was a positive correlation between the effectiveness of a plasma in lessening the parasitemia of ducklings treated with it and the resistance on infection exhibited by the duck from which the plasma had been obtained. More than half of the adult female ducks with an active ovary which were tested, but only one of the males, had effective plasmas and also showed relative resistance to the infection.

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