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Delma E. Harding  
*Iowa State College*

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## Some Responses of Chickens to Infection with *Plasmodium lophurae*

By DELMA E. HARDING

The strain of *Plasmodium lophurae*, malaria parasite, used in this investigation was one which had been received in 1947 from an infected duck supplied by the Rockefeller Institute of Princeton, New Jersey. It was maintained by transmitting it serially through young White Pekin ducks.

### METHOD

Chicken hosts were Rhode Island Red chicks brought to the laboratory just after hatching, and given a commercial chick starter. After they were five days old they were placed on a diet that was low in biotin and pantothenic acid, and kept on this for the rest of the investigation to see if the peculiar eyelid lesion phenomenon, described by Becker, Brodine, and Marousek in 1949, could be duplicated.

Blood used for inoculating the chicks was taken from the jugular vein of a passage chick which had between 70 and 80 percent of its red blood cells parasitized with *P. lophurae*. After the blood was mixed with an anticoagulant, centrifuged, and placed in a physiological salt solution, infective doses of  $0.8 \times 10^8$  infected red blood cells per 100 grams of chick body weight were injected intravenously into a wing of each chick. Inoculations were made on the twelfth day after hatching.

For determining the percentage of infected red blood cells attained in the host chicks, blood smears were made and stained with Giemsa stain each morning between 5:00 and 7:00 o'clock, starting with the first day following inoculation and continuing until the chicks had died or were sacrificed, or until no more infected red blood cells could be demonstrated in their blood. Counting was done by means of a hemacytometer.

Weight and also rectal temperature was recorded for each chick every morning, to try to determine whether any relationship seemed to exist among the three factors of parasitemia, weight and body temperature.

The following organs were removed from the bodies of the chicks, fixed in Bouin's or formol -saline, and cut into sections measuring five microns in thickness: adrenals, bone marrow, brain, eyelids, gizzard, heart, intestine, kidney, liver, lung, pancreas, rectum and spleen. Staining was done according to Tomlinson and Grocott, 1944. Imprints and smears were also made of the above mentioned organs, using Giemsa stain.

**Table 1.** Percentage of parasitized red blood cells present in infected chicks during the ten-day period following inoculation with blood stages of *P. lophurae*. Parasite dosage:  $0.8 \times 10^8$  parasitized blood cells per 100 grams body weight of chicks inoculated when 12 days old.

Chick No.	Day of the infection									
	1	2	3	4	5	6	7	8	9	10
1454	1.36	2.00	2.44	13.00	19.33	41.00	48.50*	30.00	.67	.00
1455	1.13	3.11	3.38	10.20	21.50*	18.00	18.00	.28	.00	.33
1456	1.12	2.12	2.31	Dead						
1457	Dead									
1458	1.43	1.46	1.50	6.58	16.66	16.66	17.50*	7.14	.00	.00
1459	2.11	4.63	6.60	15.25	25.50	19.21	7.86	Dead		
1460	1.32	2.54	3.33	17.57	32.00	34.50*	16.66	4.80	.00	.00
1461	.93	1.11	4.55	11.36	25.50	26.50	52.00*	48.00	12.50	.93
1462	1.14	3.13	5.15	10.00	24.50	37.50	17.00	45.50*	23.00	Dead
1463	1.21	2.00	5.44	16.66	45.00	59.00*	35.00	9.20	.36	.33
Mean	1.31	2.46	3.86	12.62	26.24	31.55*	26.57	20.70	5.22	.27

\*Day of highest parasitemia.

**Table 2.** Record of weight in grams for test chicks given inoculations with the blood stages of *P. lophurae*, on inoculation date and the ten days following inoculation. Diet for first five days was regular chick starting ration, followed by a diet low in biotin and pantothenic acid for six days prior to initiation of experiment.

Chick No.	Inoc. Date	Day of Infection									
		1	2	3	4	5	6	7	8	9	10
1454	70	67	81	79	88	93	95	104	95	102	108
1455	77	74	84	85	99	104	103	103	110	117	128
1456	77	79	86	79	Dead						
1457	Dead										
1458	70	68	81	81	92	95	98	94	96	107	119
1459	93	87	95	94	104	110	105	93	Dead		
1460	71	73	77	79	88	94	90	85	80	87	88
1461	82	79	86	89	88	88	85	93	86	84	95
1462	60	57	60	63	68	68	70	70	70	69	Dead
1463	93	93	83	83	98	100	101	85	95	108	107
Mean	77	75.2	81.4	82.8	90.6	94	93.4	91	90.2	96.3	107.5

**Table 3.** Record of weight in grams for control chicks for same days as weights taken for test group. These chicks were not inoculated with *P. lophurae*.

Chick No.	Inoc. Date	Day of infection (these not infected)									
		1	2	3	4	5	6	7	8	9	10
1472	76	78	82	87	91	98	100	105	111	115	121
1473	63	64	68	69	74	82	88	91	96	100	111
1474	86	89	94	99	103	108	111	113	115	121	128
Mean	75	77	81.3	85	88	96	99.7	103	107.3	112	120

Microscopic examinations were made of the stained slides to determine whether presence of malarial pigment could be demonstrated, to see if phagocytosis of pigment and infected red blood cells was occurring, and to learn whether phanerozoite stages of the parasite were to be found.

The present paper includes only the results shown by one group of ten host chicks, compared with a group of chicks which were not inoculated with the malaria parasite, but whose food was the same as the test group. Similar studies were made for 10 other test groups, but these results are not presented.

#### DISCUSSION AND RESULTS

1. *Parasitemia* Day of highest parasitemia ranged from the fifth day following inoculation (chicks 1455 and 1459), to the eighth day (chick 1462). See Table 1. Chick 1462 seemed unable to clear its blood of the malaria parasites, and it died on the tenth day following inoculation. Mean day of high parasitemia for the group was day six.
2. *Weight and Parasitemia* For the chicks of the test group, a general pattern between weight and parasitemia seemed to be followed. The weight dropped the day following peak parasitemia. See Tables 1 and 2. An example is shown by chick 1454 which reached peak parasitemia on day seven following inoculation. Weight on that day was 104 grams. Next day the weight recorded was 95 grams. None of the chicks in the control group showed a weight loss. See Table 3.
3. *Temperature and Parasitemia* No definite conclusions were drawn, but it was observed that in general there was a slight rise in the temperature of the chicks on the day just prior to that of peak parasitemia. The control group showed less fluctuation than did the test group. See Tables 1, 4 and 5.
4. *Malarial Pigment* The following organs showed the presence of pigment: adrenals, bone marrow, brain, eyelids, heart, kidney, liver, lung, pancreas, and spleen. Pigment appeared in the form of granules, sometimes massed together, but at other times scattered lightly throughout the organs, or in some special areas of the organs.

Figures 1, 2, 3, and 4 are photographs of four of the organs which showed the presence of pigment. No. 1 is from the spleen of chick 1456 which died on the third day following inoculation. On the day it died, a blood smear showed only a 2.31 percent parasitemia. The other three pictures are also taken of organs of the same chick. No. 2 is the liver, No. 3, a section of a lung, and No. 4 a section of the kidney, with a great amount of pigment showing near one of the kidney tubules.

**Table 4.** Temperatures recorded in degrees Fahrenheit for test on experimental chicks on date of inoculation with blood stages of *P. lophurae* and the ten days following.

Chick No.	Inoc. Date	Day of Infection									
		1	2	3	4	5	6	7	8	9	10
1454	107.6	107.4	107.6	107.6	107.7	108.1*	106.7	106.9	107.6	107.6	107.4
1455	107.0	106.8	107.0	107.3	107.9*	106.4	106.8	107.0	107.0	107.1	106.8
1456	106.7	106.5	106.0	105.3	Dead						
1457	Dead										
1458	107.0	106.7	106.6	106.8	107.6	108.0*	106.4	106.7	107.0	107.2	107.2
1459	107.0	106.8	107.0	107.1	108.6*	106.4	107.3	105.4	Dead		
1460	107.0	106.7	107.0	107.3	107.5	108.3*	107.6	107.4	107.5	107.2	107.3
1461	106.9	106.6	107.7	107.1	107.3	107.4	108.0*	105.8	106.7	107.0	106.9
1462	106.5	106.2	106.5	106.8	106.8	108.8*	106.0	106.3	106.3	104.1	Dead
1463	107.1	106.5	106.8	107.2	107.3	107.8*	105.9	106.5	106.8	106.7	106.9
Mean	107.0	106.7	106.8	106.9	107.6	107.8*	106.8	106.5	107.0	106.7	107.1

\*Day of highest temperature for each chick.

**Table 5.** Temperature changes recorded in degrees Fahrenheit for control chicks on date corresponding to inoculation of experimental chicks. Diet was the same as for the test group.

Chick No.	Inoc. Date	Day corresponding to that of experimental chicks									
		1	2	3	4	5	6	7	8	9	10
1472	106.7	106.7	106.5	107.0	106.6	106.4	106.1	106.7	106.8	107.0	107.1*
1473	107.1	107.0	106.6	106.8	106.8	107.0	107.3	106.9	107.0	106.7	107.3*
1474	107.8	107.0	101.2	106.6	107.1	107.2	107.0	107.2	107.1	107.2	107.2
Mean	107.2*	106.9	106.8	106.8	106.8	106.9	106.9	106.9	106.9	107.0	107.2*

\*Day of highest mean temperature for each chick.

5. *Phagocytosis* The spleen, liver and kidney were the best organs for observing phagocytosis. In these organs, as well as to a lesser degree in others, some of the large macrophages appeared to be filled with masses of pigment. In a number of them, phagocytosed red blood cells could be demonstrated. Figure 4, showing part of a kidney demonstrates this quite clearly.
6. *Eyelid Lesions* Four chicks of the test group showed the presence of eyelid lesions to varying degrees. A description of the appearance of lesions in both eyes of chick 1460 points out the general pattern of all which showed the lesions.

On the sixth day following inoculation, the right eye of chick 1460 appeared to be sensitive to light. The chick avoided the light side of the cage and hovered in the darkest part. When brought to the light, it closed both eyes and turned its head, appearing to shield the eyes from light. By night the right eye was watery and even more light sensitive. The left eye began to show the same signs as the right one had earlier in the day. Next morning the right lid was swollen shut. The left one was watery and the lid partly closed. Both eyes festered and the exudate formed a crust over the entire eye. Little food was taken, and there was a loss in weight.

The scab came off on the ninth day leaving a ragged margin about the ball of the eye. The chick began to eat, gained weight and a new growth of tissue developed rapidly in place of the eroded lid. A tiny, circular peep-hole was left in the right lid, and a small, horizontal slit in the other. Other chicks in the same pen pecked at the tissue frequently. It grew rapidly and became thicker than the original tissue. The chick that had the eyelid phenomenon scratched almost constantly at the new tissue until about the fifth day, when it stopped suddenly. Chick 1460 was permitted to live for nearly three months, during which time its weight was only slightly lower than that of other chicks on the same diet, but without the eyelid lesions.

7. *Phanerozoite Stages of the Malarial Parasite* Very careful examinations were made of the slides for the presence of phanerozoite stages of the malarial parasite, but all results were negative.

#### SUMMARY

1. Comparisons of weight and temperature records are made of a group of experimental chicks given infective doses of *P. lophurae*, with a group not given *P. lophurae*.
2. Records showed a constant weight gain for control chicks, but a fall in weight for chicks following the day of highest parasitemia.

3. Temperature of chicks showed a tendency to rise on the day prior to peak parasitemia, and to fall on the day following the peak parasitemia. Temperature changes were less marked in the control group.
4. Malaria pigment granules appeared in the form of small, scattered granules or in the form of large masses of pigment. Organs in which pigment were found included the adrenals, bone marrow, brain, eyelids, heart, kidney, liver, lung, pancreas, and spleen, with best organs for demonstrating this being the spleen, liver, kidneys and lungs.
5. Phagocytosis of pigment and infected red blood cells were observed in macrophages of the spleen, liver, kidneys and lungs.
6. Eyelid lesions were observed in four of the ten experimental chicks.
7. Phanerozoite stages (exoerythrocytic), were not observed in any of the organs.

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