THE TURKEY AS A HOST FOR *PLASMODIUM LOPHURAE* COGGESHALL, 1938

by

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INTRODUCTION

Malaria is still in many respects the most important single infectious disease of man. Although large areas of the world are free from malaria, it is so prevalent in the more thickly populated areas of the tropics and sub-tropics that it is probably not surpassed by any other disease in total incidence and production of illness and death.

Various workers have pointed out the need for intensifying research on the fundamental aspects of the disease, particularly with regard to the studies on chemotherapy, biology and physiology of the parasite, immunology, and the bionomics and ecology of the anopheline vectors. Since it has been shown that the results obtained from experimental studies on bird or monkey malaria often have direct application to the disease in man, birds have often been used for the study of malaria problems. The choice of a laboratory host is largely dependent upon the nature of the problem for investigation.

Chickens and ducks are generally used as hosts for Plasmodium lophurae in the United States (Hewitt, 22). Since there was very little information concerning lophurae-malaria in turkeys, a study of the biological and physiological characteristics of the parasite in this common bird seemed indicated. The present report is the first in which the host-parasite relationships of Plasmodium lophurae in turkeys

are described.

The following are some of the topics studied: (1) preliminary study on the course of infection in turkeys of various ages and infective dosage; (2) length of asexual cycle; (3) effect of host on the length of asexual cycle; (4) length and intensity of the infection; (5) temperature of the host throughout the course of the infection; (6) merozoite production, survival and death rates of the parasites during the course of the infection and during relapse; (7) immune phenomena; and (8) exoerythrocytic stages (phanerozoites).

REVIEW OF LITERATURE

The literature concerning turkeys as host for the malarial parasite, Plasmodium lopnurae, is practically nil. This review of literature, therefore, will cover only investigations that have a direct bearing on the problem under discussion, although they may be on other hosts for Plasmodium.

Coggeshall in 1938 (10) discovered Plasmodium lophurae in the blood of a Borneo fireback pheasant, Lophura igniti igniti (Shaw and Nodd), kept in the New York Zoological Park. He described the blood stages. Coggeshall found that young chicks of several breeds were quite susceptible to infection by blood-inoculation, but older chickens, though still susceptible, exhibited strong age-resistance.

Hewitt in 1942 (22) also published descriptions of Plasmodium lophurae and illustrated the blood stages of the parasite in colored plates. He observed some 300 ducks of mixed ages, weights, and breeds, and found that over 75 per cent of 30 young ducks infected when 4 to 7 weeks old died from what appeared to be the effects of the parasites, and that infections in 10 to 15 per cent of about 250 adult ducks terminated fatally. His observations on rectal temperatures showed an average of about 107.4°F $\pm$ 0.14 for unparasitized normal ducks, with increase to 110° or 111°F during infection, and that subnormal temperatures usually

preceded death, or followed high peaks of parasitemia. From the study of blood smears of 6 birds made at 4-hour intervals throughout the course of infection, Hewitt found that the asexual cycle of Plasmodium lophurae in ducks averaged about 36 hours in length, and that the parasite exhibited asynchronous reproduction. His asexual cycle estimate was based on the time interval between the peaks and troughs of any one stage.

The relations between infection and body temperature were studied in canaries by Huff in 1939 (24). He found no temperature rises in canaries accompanying the periods of greatest segmentation of the asexual forms of the avian malarial parasites. No significant differences were found in the mean pectoral muscle temperature for the birds infected with the M strain (Plasmodium cathemerium), R strain (Plasmodium relictum matutinum), and C strain (Plasmodium relictum), and for the uninfected birds. These mean temperatures were, respectively, 41.53°C ± 0.28, 41.66°C ± 0.31, 41.78°C ± 0.13, and 41.73°C ± 0.24. The range of temperature in individual birds was sometimes as much as 6°C over a 24-hour period. The mean day temperature was 42.3°C ± 0.05 and the mean night temperature was 41.0°C ± 0.26 in uninfected birds.

Baldwin and Kendeigh in 1932 (1) made a comprehensive study on the physiology of temperature in birds. They found

the body temperature in 5 species of passiform birds to be about 104.4°F in males and 105.0°F in females.

Hewitt, Richardson, and Seager (23) in 1942, found that of 146 ducks which had been inoculated with 2 billion parasites per kilogram, with no subsequent treatment, 21.9 per cent reached their peak on the 4th day after inoculation, 53.4 per cent on the 5th day, 21.8 per cent on the 6th day, and 2.8 per cent on the 11th day. They considered, therefore, that parasite counts made on these 4 days would include the peak of parasitemia in nearly 100 per cent of the birds.

In 1941 Terzian (43, 44) made studies of the biological characteristics and host-parasite relationships of Plasmodium lophurae in chickens. He reported the following: (a) the morphological characteristics of the organism were in accordance with the description given by Coggeshall (1938); (b) studies made from blood films taken at intervals varying from 2 to 6 hours during the periods of observation of from 38 to 96 hours, demonstrated periodicity by the regularity in the number of hours between successive peaks of segmentation in the same bird, and by the coincidence of such peaks in different birds; (c) although the degree of synchronicity was shown to be low, the length of the asexual cycle was established as 48 hours; (d) pathological changes taking place were similar to those described for other avian malaria observed in canaries, for blood examinations showed the degree of red cell destruction to correspond roughly to the

number of parasites present during the infection, and that red cell regeneration took place very rapidly in recovered chicks; (e) results of experiments in which parasitized cells were washed free of serum indicated that parasites were unaffected by this treatment and that the presence in the inoculum of serum which might contain immune bodies had no effect in either inhibiting the organism or enhancing its aggressive powers in the new host.

In a study of avian host for blood-induced lophurae-malaria research in 1940 and 1941, Wolfson (47, 48) first noted the high susceptibility of ducks as evidenced by high and often fatal parasitemias.

Becker, Brodine, and Clappison (3), as well as a number of other workers, reported that the parasitemias induced in young ducks by moderately heavy intravenous injections of parasitized cells (such as, 1.5 x 10^8 per 100 grams body weight) tend to reach the peak on the fifth or sixth day, then decline precipitously for two or three days, and less precipitously for another two or three days. Young untreated ducks succumb within three weeks to this primary attack in 90 - 95 per cent of the cases (Marshall, Litchfield, and White, 1942, (34)).

Becker, Marousek and Brodine (6) in their observations of Plasmodium lophurae infection in ducklings showed that actively acquired resistance in lophurae-malaria was not

resident wholly or in part within the red blood cells, but it was concerned with other tissues; that immature red blood cells of the duck are more suitable for host for Plasmodium lophurae than had been previously appreciated; and that the decline in incidence of parasitized red blood cells following the peak of the parasitemia cannot, therefore, be attributed in any considerable part to the aversion of the parasite for red blood cells.

Regarding post-crisis developments, Becker, Marousek, and Byrd (4, page 323), in 1949 wrote the following:

> It is now generally accepted by malariologists that the immune state following parasitemia, during which malarial parasites are either not microscopically demonstrable in the circulating blood or are detectable only after prolonged search, is a premunition, or an immunity that is dependent upon more or less constant stimulation of the defense mechanism of the host by uneradicated residue of parasites. Such an immune state is characterized by refractoriness of the host to reinoculation with homologous strain of malarial organisms, the appearance in the circulating blood of antibodies that can be demonstrated if suitable test are made at the proper time, and the ability of the blood of the latently infected host to produce acute infection in normal animals by subinoculation. These statements hold whether or not exo-erythrocytic forms occurs in the host.

Coggeshall in 1940 (11) presented evidence which indicated that antibodies appeared in the serum of individuals following an induced malaria infection with Plasmodium knowlesi.

The research in Rhesus monkeys with experimental

Plasmodium knowlesi infections by Maier and Coggeshall in 1944 (33) showed that in monkeys which survived acute infection with the aid of immune serum or quinine and in which a naturally acquired immunity had developed to the point where the acute infection was converted into a chronic one, there was an undoubted persistence of partial immunity up to about a year after sterilization of the infection with sulfathiazole, as indicated by recovery of reinoculated animals after mild or moderately severe infections differing widely in characteristics from the infections with the normal monkey. The end point at which immunity disappears seems to be independent of the length of the chronic infection.

Taliaferro and Taliaferro in 1940 (37) in their work on immunity in chickens against Plasmodium lophurae showed that ordinarily latency was not intercepted by relapses, but that the parasites can be demonstrated for 4 months (the longest period tested) by the sub-inoculation of large quantities of blood into normal chickens. Asexual reproduction lacked synchronicity. They further reported the well-defined age immunity to the infection and the fact that acquired immunity can be passively transferred to normal chickens provided sufficient doses of immune serum (from latently infected chickens) are used and continued over a sufficient period. In successful experiments serum was given for nine days which

included the period of the acute rise of the infection.

Becker, Brodine, Marousek, and Byrd in 1949 (4) discovered the "sparing action" phenomenon in duck plasma. The term "sparing action" was applied by the authors to the protective influence exerted by the treatment in behalf of the parasitized duck erythrocytes injected into the chick. The term was adopted in preference to "protection" in order to avoid confusion with protection afforded the host by treatment. The foregoing authors gave the following conclusions: (1) immune and normal duck plasmas may exert a sparing action on the removal of duck erythrocytes, parasitized with Plasmodium lophurae, from the peripheral circulation of chicks; (2) plasma from ducks recovered from the primary attack may confer a passively acquired partial protection on chicks inoculated with parasitized cells; (3) plasma from normal ducks may also exert a similar protective effect; (4) plasma taken from ducks in relapse may exert an initial sparing effect on the removal of duck erythrocytes from the blood of injected chicks, or exhibit extremely potent antibody properties almost from the very start. (The latter difference in behavior is, presumably, dependent on whether or not the host has reacted to the recrudescense of the parasitemia); (5) and that the interrelationships of the aforementioned sparing action of duck plasma and concentration of antibody in duck blood constitute a problem that

may have important implications in the problem of the nature of relapse in lophurae-malaria.

Becker, Schwink, Byrd, and Conn in 1950 (8) reported results of additional experiments which provided further and incontrovertible proof of the sparing effect of normal and immune duck plasmas on the duck erythrocytes parasitized with Plasmodium lophurae against destruction when injected into the blood stream of young chicks. They concluded that it was the chick's innate resistance to the introduced parasitized red cells that is affected in the sparing phenomenon, and that the property of the bird plasma producing the sparing effect may be an inhibitory antibody, though other plausible explanations also suggested themselves.

The work of Becker, Schwink, and Prather in 1951 (9) on hemagglutinin inhibition by duck plasma, reproduced what may prove to be the in vitro counterpart of the sparing phenomenon, viz., inhibition of hemagglutinins for parasitized duck erythrocytes in the plasma of chicks recovered from lophurae-malaria. They pointed out that the cause of relapse in malaria should be sought in the area of the interplay of sparing and so-called protective (i. e., hemagglutinin, opsonin, hemolysin) factors.

Becker, Schwink, and Brodine in 1951 (7), in another study of sparing action, concluded as follows: (1) the bulk of the sparing factor in immune duck plasma is con-

centrated in the protein fraction precipitated by ammonium sulphate in the 33.4 per cent to 50 per cent saturation range; (2) the bulk of the factor that confers a degree of passive immunity is concentrated in the protein fractions precipitated by ammonium sulphate in the 0 per cent to 33.3 per cent saturation range; (3) the supernatant over the precipitate at 50 per cent to 51 per cent saturation with ammonium sulphate is relatively inactive in respect to both sparing and protective activities; (4) by selecting samples on the basis of the results of tests it is possible to obtain fractions of plasma potent in protective factor and weak or lacking in sparing factor; and vice versa; and (5) the therapeutic value of fractions of plasma which are fortified in protective antibody as well as denatured in respect to sparing factor constitutes an interesting problem that requires further elucidation.

Modification of Plasmodium cathemerium when transferred from canaries into ducks was studied by Hegner and West in 1940 (20). The prepatent period of Plasmodium cathemerium W strain in canaries ranged from 3 to 5 days; in ducks it ranged from 3 to 8 days depending on the age of the bird and the number of parasites inoculated. The greater length of the prepatent period appeared to be due to the greater amount of blood in the ducks. The patent period of the H-H strain in canaries was from 8 to 14 days, although usually about 60 per cent of the birds died from

this virulent strain. In 3 ducks the patent period was 11 days in each. Synchronicity was higher in canaries than in ducks, as revealed by the counts of segmenters and rings at 4-hour intervals, and in ducks it became lower from day to day. Segmentation was delayed in ducks. No change in the length of the asexual cycle was observed. Canaries infected with the H-H strain exhibited severe symptoms and pathology, while ducks did not exhibit symptoms, probably because of the small number of parasites. In both birds the spleen became pigmented and enlarged. No difference was noted in the position of young parasites in the red cells. Red cells containing segmenters were smaller than normal in both canaries and ducks. The number of merozoites produced by segmenters were greater in canary cells than in duck cells.

Hegner and West in 1941 (21) transferred a malaria parasite, Plasmodium cathemerium, from canaries or ducks to fowls. They found the asexual cycle to be lengthened from 24 hours in canaries and in ducks to 48 hours in chicks. The time of day when segmentation occurred also changed. The red cells in chicks were the same in breadth but slightly larger than in canaries and not as long as in ducks. The number of merozoites in chick cells was much smaller than in canary or duck cells.

The classic discovery by James and Tate in 1939 (32)

that certain developmental stages of Plasmodium gallinaceum in chickens, to which they gave the name exoerythrocytic stages, appeared outside of erythrocytes, opened a new field of study in malariology.

The advance of knowledge concerning exoerythrocytic stages has been greatly accelerated due to the importance of malaria in World War II. In 1948, a symposium was held on exoerythrocytic forms of malarial parasites, wherein Huff (27) stated that exoerythrocytic stages were well-known only in certain species of Plasmodium parasitizing birds and in one species parasitizing lizards. In Plasmodium elongatum of birds almost all cells of the blood and the blood-forming cells of the host are parasitized, but the cells of the erythroblastic series are preferred (Huff and Bloom, 28). In all the other species of avian malaria which have been adequately studied the cells of the lymphoid-macrophages system are preferred, although true endothelium and some other types of cells may be parasitized. Thompson and Huff in 1944 (45) reported that in Plasmodium mexicanum of lizards the potencies of Plasmodium elongatum and of the species mainly inhabiting the lymphoid-macrophage cells are combined, with the result that any of the cells of the lizard belonging to either of these categories may be parasitized.

Detailed accounts of the history of the development of

the knowledge on exoerythrocytic stages have been published by Porter and Huff in 1940 (35), Huff and Coulston in 1944 (29), and Huff in 1947 (25).

The term phanerozoites was proposed by Huff and Coulston in 1946 (30) for the exoerythrocytic stages of malaria parasites which occur late in the infection and not as one of the pre-erythrocytic stages. The latter were called cryptozoites or metacryptozoites.

A list of the species of Plasmodium in which exoerythrocytic stages had been demonstrated was published by Huff in 1948 (26). The complete development from sporozoite to erythrocytic trophozoite was demonstrated in Plasmodium gallinaceum by Huff and Coulston in 1944 (29). Since then similar development of the pre-erythrocytic stages of Plasmodium cathemerium and Plasmodium relictum has been reported by the same author of Plasmodium lophurae (27), and in collaboration with Laird and Porter (31). Phanerozoites are known in Plasmodium circumflexum, P. durae, P. mexicanum, and P. elongatum, according to the listing of Huff (26).

Becker and Manresa in 1950 (5) found phanerozoites in brain smears of two 46-day-old turkeys which succumbed to infection with Plasmodium lophurae.

In turkey poults and young pheasants, Huff, Coulston, Laird, and Porter in 1947 (31) found that small doses of sporozoites gave intense infections and abundant exo-

erythrocytic stages in brain capillaries.

Taliaferro and Taliaferro in 1950 (42) in their research concerning reproduction-inhibiting and parasiticidal effects on Plasmodium gallinaceum and Plasmodium lophurae during initial infection in chickens, concluded that initial infections of Plasmodium gallinaceum and Plasmodium lophurae had the same length of asexual cycle, 36 hours, but that they differed markedly with respect to synchronism of the cycle, rate of reproduction, and survival and death of parasites. Their methods of differentiating reproduction-inhibiting and parasiticidal effects of immunity have been fully described in earlier papers (38, 40, 41).

MATERIALS AND METHODS

This discussion will be covered in parts. First, the general procedures which were used in the experiments will be described. Then each set of procedure for a particular experiment will be discussed in a general way leaving details which will be covered in the treatment of results and in the discussion.

General

Strain of Plasmodium lophurae

The strain of Plasmodium lophurae used in the experiments was one which Dr. E. R. Becker had maintained in ducks by blood passaging since 1947. It was originally obtained from an infected duck supplied by Dr. William Trager of the Rockefeller Institute of Princeton, New Jersey, in 1947. Since that time the parasite has been transmitted serially through ducklings by intravenous injections of moderately heavy doses of parasitized cells at intervals of four, five or six days, with the white Pekin duck as host.

Turkeys used

The turkey used in the experiments was of the bronze broad-breasted type. The turkey poults employed as host in the experiments were obtained from the hatchery when one day to a week old. The ration was a commercial chick starter

which had been found satisfactory for proper chick development (see Appendix).

Method of inoculation

The source of parasitized cells was blood drawn from the jugular vein of a duck or turkey when about 70 - 80 per cent of its erythrocytes were parasitized. In the duck that was about the 4th, 5th, or 6th day, and in the turkey about the 5th, 6th, or 7th day of infection. The drawn blood was placed in a tube containing an anticoagulant (1/2 cc of 0.8 per cent sodium citrate dried in the bottom of a 15 cc glass tube). After mixing the anticoagulant, about 5 cc of blood in a screw-cap test tube was whirled at about 2,000 r.p.m. for about 5 minutes in the centrifuge, then the supernatant plasma was removed. About 10 cc physiological salt solution was added to the cell residue and the mixture was shaken to wash the red cells and centrifuged again for 5 minutes. Three or four such washings were done, and after the last washing the red blood cells were resuspended in the amount of physiological salt solution that was required for the desired concentration of inoculum. The computation for obtaining this concentration was based on parasite counts on stained, dried blood smears and total erythrocyte counts made on the infected host just before the blood was drawn. The method used for total erythrocyte counts was the same as those described by Beck (2) and by Wintrobe (46).

The blood plasma and parasitized erythrocytes were injected into the wing veins. Parasite dosage are expressed in terms of 10^8 per 100 grams of the turkey poult's body weight and the plasma dosage as cc per 100 grams of the poult's body weight.

Other general procedure

The criterion of the effects of the various treatments in the experiment was the percentage of parasitized erythrocytes in control and injected groups at appropriate intervals of time. The cell counts were made on dried blood smears stained in Giemsa. The number of cell counted was large enough to give a parasite-erythrocyte ratio with a probable error of 10 per cent (Gingrich, (16); see Appendix).

Age of Turkeys and Infective Dose

In view of the lack of studies on the age of turkeys and infective dose for use in lophurae-malaria experiment, preliminary study on the subject was conducted. Turkey poults of various ages ranging from 6 to 32 days were infected with different doses of the parasites. The intensity of their parasitemias was observed. Details of the experiment are described more fully under "Results and Discussion."

Host-Parasite Relationships and Biological Characteristics

The effect of the host on the asexual cycle of the parasite was determined by transferring the infection from the duck to the turkey, then from turkey to turkey for two further passages. To ascertain the length and synchronicity of the asexual cycle, differential counts were made of parasites on dried smears stained in Giemsa. The smears were made at intervals of 3 hours for a period of 72 to 98 hours during the peak of infection. The study consisted of classifying 50 to 100 parasites taken at random into 5 groups of (1) ring stages, (2) old trophozoites, (3) parasites with 2 to 5 nuclei, (4) parasites with 6 or more nuclei, and (5) segmenters. Time interval between ring stages was the criterion for determining the length of the asexual cycle of the parasite.

Studies on temperature were conducted in two series of turkeys, test and control. Temperatures were taken at 3-hour intervals for the duration of the infection by means of a thermometer inserted into the rectum.

Experiments on merozoite production and survival and death rates of the parasites formed during the initial infection, as well as in relapse, were conducted by making blood smears on individual bird at 3-hour intervals. Twenty-five turkeys were infected and blood smears were made on them for 20 days. A daily parasitemia count was

made on each bird and the reproduction rate and survival and death rates determined only in birds that relapsed.

Latency experiments were also conducted by injecting clean birds with heavy dosages of blood from turkeys which survived acute malaria infection and whose blood exmination remained negative for a period of time.

Immunity Experiments

The immunity experiments consisted of two sets of trials. In the first set, both duck and turkey immune plasmas were tested, while only the duck plasma was tested in the second set.

The plasma or serum used for these two sets of experiments was obtained from blood drawn from the jugular vein of immune duck or turkey by means of a syringe. The drawn blood was transferred to screw-cap glass test tubes containing sufficient anticoagulant (1/2 cc of 0.8 per cent sodium citrate per 15 cc blood). After mixing with the anticoagulant, the tube was centrifuged for 30 minutes, after which the plasma was pipetted off. The plasma was then transferred to another screw-cap glass test tube with a serum preservative (1 cc of phenyl mercuric nitrate solution to 40 cc serum), then kept in a refrigerator.

Blood plasma was administered an hour before injecting the parasitized red cells, then afterwards on the 1st, 2nd,

3rd, and 4th days with doses of 0.8 cc per 100 grams body weight of the bird.

All counts were made on blood smears made at 10 minutes, 6 hours, 12 hours, 1 day and then daily up to 11 days after infection. Smears were made every other day after the eleventh day.

Exoerythrocytic Stages

In conjunction with all the experiments herein discussed, the poults that died were examined for exoerythrocytic stages. Turkeys that survived the parasitemia were killed at various intervals and also examined for exoerythrocytic forms. Tissue smears of the brain, spleen, liver, kidney, heart, and lung were stained in Giemsa.

A system for determining the intensity of exoerythrocytic infection was devised in the absence of any system now being used. Details of this system will be discussed under "Results and Discussion."

RESULTS AND DISCUSSION

For convenience of reference, the results of the experiments and their discussion are placed in one section. The experiments will be presented and discussed under the same divisions as in the section "Materials and Methods."

Age of Turkeys and Infective Dose

Since there have been no previous experiments on this subject in turkeys, preliminary experiments were conducted to observe the course of infection using various ages of turkey poults and also to determine a satisfactory dosage of parasitized cells for inoculation. The experiments were not designed for critical testing of the effects of either the age of hosts in the infection or dosage of inoculated parasites but only for indications. Four trials were conducted using poults varying in age from 6 to 32 days and doses varying from 0.5×10^8 to 2.6×10^8 parasitized cells per 100 grams body weight of the birds.

The results of the above series are shown in Tables 1 - 4, and summarized in Table 5.

Table 1

Percentage of Parasitized Cells in Infected 6-Day-Old Turkey Poults Inoculated With 2.6×10^8 Parasitized Duck Cells (Series 1; Average Weight, 69.4 g.)

Time After Infection	Designations of Turkey Poults					
	52	53	54	55	56	Average
15 Min.	0.56	0.27	0.16	0.40	0.50	0.38 ± 0.16
1 Day	0.06	0.03	0.16	0.63	0.36	0.25 ± 0.24
2 Days	0.06	0.13	0.20	0.33	1.26	0.40 ± 0.48
3 Days	3.80	1.10	1.76	3.92	11.75	4.46 ± 4.25
4 Days	19.00	7.14	7.28	37.50	28.50	19.88 ±13.26
5 Days	11.80	10.77	9.77	46.50	38.88	23.37 ±19.33
6 Days	62.00	55.50	42.00	80.00	41.50	56.20 ±15.94
7 Days	70.00	55.00	45.50	54.00	3.12	45.52 ±25.28
8 Days	75.00*	85.00*	80.00*	40.00**	3.80	56.76 ±33.47

*Died.

**Killed for inoculation transfer.

Table 2

Percentage of Parasitized Cells in Infected 14-Day-Old Turkey Poults Inoculated With 1.6×10^8 Parasitized Turkey Cells (Series 2; Average Weight, 116.0 g.)

Time After Infection	Designations of Turkey Poults					
	93	94	95	96	97	Average
15 Min.	0.23	0.26	0.20	0.13	0.23	0.21 ± 0.05
1 Day	0.43	0.30	0.26	0.13	0.60	0.34 ± 0.17
2 Days	2.26	2.60	1.30	1.00	2.80	1.99 ± 0.79
3 Days	22.70	18.33	7.60	7.25	28.00	16.77 ± 7.04
4 Days	34.50	41.00	16.00	9.00	43.50	28.80 ±25.55
5 Days	63.50	68.00	51.00	42.50	77.00	61.40 ±13.68
6 Days	60.00*	80.00*	80.00**	75.00**	66.00*	72.30 ± 7.82

*Died.

**Killed for inoculation transfer.

Table 3

Percentage of Parasitized Cells in Infected 25-Day-Old Turkey Poults Inoculated With 0.5×10^8 Parasitized Turkey Cells (Series 3; Average Weight, 220.0 g.)

Time After Infection	Designations of Turkey Poults					
	1	2	3	4	5	Average
15 Min.	0.10	0.20	0.13	0.03	0.07	0.11 ± 0.02
1 Day	0.37	0.30	0.27	0.50	0.53	0.39 ± 0.12
2 Days	1.27	0.93	1.00	2.05	1.00	1.25 ± 0.47
3 Days	7.62	5.40	9.20	5.55	7.00	6.95 ± 2.04
4 Days	25.50	32.50	26.00	26.00	20.00	26.00 ± 4.43
5 Days	46.00	31.00	44.50	36.50	39.50	39.50 ± 5.12
6 Days	56.00	57.00	72.00*	40.00	43.50	49.00 ±12.65
7 Days	13.50	21.70**		9.22	8.00	13.10 ± 6.19

*Died.

**Killed for inoculation transfer.

Rest of turkeys lived through the infection.

Table 4

Percentage of Parasitized Cells in Infected 32-Day-Old Turkey Poults Inoculated With 0.5×10^8 Parasitized Turkey Cells (Series 4; Average Weight, 384.5 g.)

Time After Infection	Designations of Turkey Poults				
	6	8	9	10	Average
15 Min.	0.13	0.16	0.10	0.20	0.14 ± 0.04
1 Day	0.80	0.73	0.53	0.90	0.76 ± 0.15
2 Days	2.58	2.43	2.75	4.25	3.00 ± 0.83
3 Days	15.50	23.50	25.00	25.00	22.25 ± 4.55
4 Days	63.00	73.00	66.00	75.50	69.37 ± 5.85
5 Days	85.00	75.50	68.50	82.00	77.75 ± 7.33
6 Days	86.00*	90.00*	86.00*	87.00*	87.35 ± 1.89

*Died.

Table 5

Summary of Results in Series 1 to 4 of the Mean Percentages of Parasitized Erythrocytes Made on Selected Days (Minutes When Indicated) After Inoculation; Ages; Inoculation Doses; and Mean Weights of Turkeys

Item	Series Number 1	2	3	4
Age in Days	6.00	14.00	25.00	32.00
Ave. Wt., g.	69.40	116.00	220.00	384.50
Ino. Dose, P.C. per 100 g. Body Wt.	2.6×10^8	1.6×10^8	0.5×10^8	0.5×10^8
Time After Inoculation:				
15 Min.	0.38 ± 0.16	0.21 ± 0.05	0.11 ± 0.02	0.14 ±0.04
1 Day	0.25 ± 0.24	0.34 ± 0.17	0.39 ± 0.12	0.76 ±0.15
2 Days	0.40 ± 0.48	1.99 ± 0.79	1.25 ± 0.47	3.00 ±0.83
3 Days	4.46 ± 4.25	16.77 ± 7.04	6.95 ± 2.04	22.25 ±4.55
4 Days	19.88 ±13.26	28.80 ±25.55	26.00 ± 4.43	69.37 ±5.85
5 Days	23.37 ±19.33	61.40 ±13.68	39.50 ± 5.12	77.75 ±7.33
6 Days	56.20 ±15.94	72.30 ± 7.82	49.00 ±12.65	87.35 ±1.89
7 Days	45.52 ±25.28		13.10 ± 6.19	
8 Days	56.76 ±33.47			

In series 1 (Table 1), the five 6-day-old poults received inoculation doses of 2.6 x 10^8 parasitized duck erythrocytes. It may be noted that unlike the other three series, which were inoculated with turkey parasites, the average percentage parasitized cells decreased from 0.38 per cent on the 15 minutes count, to 0.25 per cent on the 1st day count. There was a gradual rise in parasitemia from the 1st to the 6th day of infection when the peak occurred, followed by a decrease on the 7th day due mainly to 1 bird, No. 56, which dropped suddenly to 3.12 per cent in parasitemia. On the 8th day, 3 birds died when they had a parasite count of 75, 85, and 80 per cent.

Series 2, 3, and 4 are similar in that they were all infected from turkey parasitized erythrocytes. The ages of the poults were 14 days in the 2nd series, 25 days in the 3rd series, and 32 days in the 4th series, and dosage of inoculum were 1.6 x 10^8, 0.5 x 10^8, and 0.5 x 10^8 parasitized cells per 100 grams body weight, respectively.

In the 2nd series the peak was reached on the 6th day of infection at which time 3 of the 5 turkeys died with parasitemia counts of 60, 80, and 66 per cent. The 3rd series reached its peak of infection also on the 6th day (see Table 3). Death due to erythrocytic infection occurred in turkey 3 while the rest lived through the infection. While there were more birds that survived in the 3rd series, it may be noted that the dose of infection was much less

than that in series 2.

The results of series 4 (Table 4) showed that older 32-day-old poults seemed to be just as susceptible to infection as are young poults. In this series the peak was reached on the 6th day of infection. All the birds died when their percentages of parasitized cells were 86.00, 90.00, 60.00, 87.00, and 87.35 percent, respectively. It may be noted that this series received the same dose of parasitized cells as series 3, 0.5×10^8.

The results obtained in the four series of experiments are summarized in Table 5. From the table it may be noted that the age range of poults used was from 6 to 32 days old. Younger or older birds of this age range were not tested as their sizes proved unsuitable for use in the present experiments.

While the results of the preliminary trials on age and infective doses indicate that turkeys up to 32 days old do not show age resistance, turkeys of about 14 to 25 days old proved to be a most suitable size for use in the present experiments. Birds of this size are more easily handled, both in inoculation and maintenance, and are more economical in the amount of blood plasma required for each bird on the tests.

Similar results have been found in ducks by Wolfson (48) in 1941. She reported that her studies showed that the duck

exhibited no age immunity to infection with Plasmodium lophurae.

Coggeshall (10) in 1938, however, found that young chicks of several breeds were quite susceptible to infection by blood-inoculation, but that older chickens, although still susceptible, exhibited strong age resistance.

Host-Parasite Relationships and Biological Characteristics

Morphology

The morphological characteristics of Plasmodium lophurae in the turkey was found to be in accordance with the descriptions given by Coggeshall in 1938 (10) for the parasites in the chicken and by Hewitt in ducks (1942, 22).

Immature erythrocytes during primary infection

It has been observed in the preliminary experiments that numerous immature red blood cells appeared during the course of the primary infection of Plasmodium lophurae in turkeys. Soon after their appearance a large number of them became infected with the parasite. To determine the preference of the parasite for mature or immature red blood cells, counts of the percentage of parasitized erythrocytes, immature red blood cells, and infected immature red blood cells, were made in three groups of infected turkeys. Since the data herein obtained for this study were from the same groups of

turkeys used in the study of immunity to follow, the groups were designated as the duck plasma-recipients, the turkey plasma-recipients, and the control (untreated) group.

The percentage of immature red blood cells of a particular sample was estimated by counting the immature and mature red blood cells in a total of 200 red blood cells. Red blood cells were considered mature when the cytoplasm was orange in color with the Giemsa stain, and they were classed as immature when the cytoplasm was either bluish or grayish in color. Other differences used to differentiate between immature and mature red blood cells were the same as those used by Hegner and Eskridge in 1938 (19): (a) certain young types of cells are larger than older cells; (b) the nucleus of younger cell is larger and the chromatin is scattered about within it in rather large clumps, whereas, in old cells, the nucleus is visibly smaller and the chromatin forms a homogeneous mass.

The results of the counts of the total percentage of parasitized red blood cells, percentage of immature red blood cells, and percentage of immature infected red blood cells for each of the three groups under study appear in Table 6. The data represent the average of 7 turkeys for the duck plasma recipients, 6 turkeys for the control group, and 7 turkeys for the turkey plasma recipients, at various times after inoculation.

As shown in Table 6, the production of immature erythro-

Table 6

Records of the Percentages by Groups of the Total Red Blood Cells, Immature Red Blood Cells, and Infected Immature Red Blood Cells (Age, 11 Days; 2.5 x 10^8 P. C.; P. C. = Total Parasitized Cells; I. E. = Immature Erythrocytes; I. I. E. = Infected Immature Erythrocytes)

Time After Inoculation	Duck Plasma - Recipients			Control			Turkey Plasma - Recipients		
	P. C.	I.E.	I.I.E.	P.C.	I.E.	I.I.E.	P.C.	I.E.	I.I.E.
10 Min.	0.70	5.07	0	0.39	5.83	0	0.35	6.21	0
1 Day	0.78	7.14	0	0.13	9.92	0	0.10	8.07	0
2 Days	3.27	12.43	2.64	0.37	18.17	0	0.54	14.71	0.50
3 Days	7.76	20.86	5.07	1.87	25.17	0	1.53	20.64	1.64
4 Days	26.78	16.43	3.64	7.65	17.17	2.08	6.28	15.50	0.64
5 Days	38.30	12.79	5.50	8.70	15.17	6.08	6.47	14.64	3.78
6 Days	45.85	33.00	4.71	27.16	23.25	2.17	19.30	19.71	1.71
7 Days	33.85	50.43	55.00	48.58	31.75	12.17	25.52	36.29	28.85
8 Days	10.71	46.36	1.14	34.83	28.08	2.83	18.68	24.35	2.43
9 Days	7.98	41.21	3.43	16.74	35.17	4.92	16.87	26.42	2.57

Table 6 (Continued)

Time After Inoculation	Duck Plasma - Recipients			Control			Turkey Plasma - Recipients		
	P.C.	I.E.	I.I.E.	P.C.	I.E.	I.I.E.	P.C.	I.E.	I.I.E.
10 Days	0.91	18.36	1.71	5.98	37.67	2.16	5.62	22.64	0.78
11 Days	0.25	15.71	0.28	2.74	24.58	7.50	1.83	18.36	0.50
13 Days	0	8.16	0	1.12	17.20	0	0.06	12.50	0
15 Days	0.02	4.91	0	0.10	7.20	0	0.08	6.00	0

cytes in all three groups commenced as early as the 2nd day and reached the peak immature cells shortly after the crisis in parasitemia on about the 6th or 7th day. Similar studies of Hewitt in 1942 (22) showed that when the red blood cells are destroyed by the liberation of merozoites from mature segmenters, immature erythrocytes are liberated into the peripheral blood from the centers of erythropoietic activity.

There was a gradual decline in immature red blood cell production from the 6th or 7th day after infection until the total erythrocyte count returned to normal towards about the 15th day after infection.

Terzian (44) in 1941 in his work on Plasmodium lophurae reported that red cell regeneration took place very rapidly in recovered chicks and the counts usually returned to their normal values 5 days after the parasite had disappeared from the circulating blood.

It is to be noted that the incidence of parasitized cells (P. C.), both mature and immature, from time intervals of 10 minutes and up to 15 days after inoculation, varied from 0 - 45.85 per cent in the duck plasma-recipients, 0.10 - 48.58 per cent in the control group, and 0.06 - 25.52 per cent in the turkey plasma-recipients. The incidence of parasitism in the immature erythrocytes for the same groups and periods varied from 0 - 55.00 per cent, 0 - 12.17 per cent, and 0 - 28.85 per cent, respectively. It is also in-

teresting to note that on the 7th day, when the incidence of immature erythrocytes was 50.00 per cent in the duck plasma-recipients, 31.75 per cent in the control group, and 36.29 per cent in the turkey plasma-recipients, 55.00 per cent, 12.17 per cent, and 28.85 per cent, respectively, of the immature red blood cells were parasitized. There were actually more parasitized young red cells in the blood of the duck plasma-recipients on the 7th day than parasitized adult erythrocytes. A similar incidence occurred in the controls on the 11th day and in the turkey plasma-recipients on the 3rd and 7th days.

This result, in turkeys, on the preference of Plasmodium lophurae for immature erythrocytes is in accordance with the finding of Becker, Marousek, and Brodine in 1949 (6) wherein they found that immature red blood cells of the duck are more suitable hosts for Plasmodium lophurae than had previously been appreciated. Their results are also in agreement with those of Hegner and Eskridge in 1938 (19) for three species of avian Plasmodia, P. relictum, P. circumflexum, and P. elongatum, using as host the canary, pigeon, chick, and red-winged blackbird.

That Plasmodium cathemerium and Plasmodium relictum preferred mature erythrocytes, whereas Plasmodium lophurae, whether residing in chick or the duck, preferred mature erythrocytes, was reported by Wolfson in 1941 (48).

Latency

In the usual course of malarial infection in chickens and ducks, in which the host survives, shortly after the bird passes the critical stage of the disease (the crisis) the parasites disappear from the circulating blood and can no longer be demonstrated by the usual microscopic methods or are detectable only after prolonged search. The parasite can be demonstrated, however, by producing parasitemia in birds transfused with blood from the latent birds.

In an effort to determine whether the parasite still persisted in the blood of recovered turkeys, two turkeys which had become negative on blood smear examinations for Plasmodium lophurae after recovery from the primary infection were used as test animals. Twenty-five cubic centimeters of blood were withdrawn from each, and 10 cc was injected into each of four 14-day-old normal turkeys. The longest period of latency tested was 97 days, the other 45 days.

The blood of both turkeys produced acute infections in the normal turkeys. The results show that a latency period similar to that in other avian host occurs also in turkeys. Taliaferro and Taliaferro (37) in 1940 working on Plasmodium lophurae in chickens reported that inoculation with a large number of parasites produced an infection which climaxed in a sharp crisis followed within a few days by latency. Or-

dinarily, latency in chickens is not interrupted by relapses, but the parasites could be demonstrated for 4 months (the longest period tested) by sub-inoculation of large quantities of blood into normal chickens. In 1950 the same authors (42) reported that the infection lasted during at least 1 1/2 years of latency.

Becker, Brodine, and Clappison (1949, 3) in their study of the post-crisis in Plasmodium lophurae infections in White Pekin Ducks, found it possible to divide the post-crisis infections into four types, of which the latent apparently was the rarest.

Effect of the host on asexual cycle: length of asexual cycle, synchronicity, and periodicity

A number of studies using a variety of methods have been made on the periodicity, synchronicity, and the length of asexual cycle in avian malaria. Observations on these topics were made in this study as part of the work on the effect of the host on the asexual cycle.

The length of the asexual cycle was first determined in passage ducks. This particular strain has been maintained by blood passage in ducks in this laboratory since 1947, the transfers to clean ducks being made every 5 to 9 days. Blood smears were made at 3-hour intervals on days when the parasite counts were at their maximum. Counts and smears were made at this stage to assure sufficient numbers to

facilitate the counting. Having determined the length of the asexual cycle in the duck, the parasitized duck blood was passed to turkeys (duck-turkey passage), then from this bird another transfer was made to turkeys (1st turkey-turkey passage), and lastly to other turkeys (2nd turkey-turkey passage). The length of asexual cycle was determined in each host. Observations were made also on the periodicity and synchronicity of the parasite in the turkey as host.

To determine the length of the asexual cycle, periodicity, and synchronicity of the parasite, one hundred asexual forms were taken in consecutive order as they appeared in successive fields on the slide. The organism were grouped into 5 separate developmental classes as follows: (1) ring stages, (2) old uninucleate trophozoites, (3) presegmenters with 2 to 5 nuclei, (4) presegmenters with 6 or more nuclei, and (5) mature segmenters.

Two series were conducted in the above experiment on the effect of the change of host on the asexual cycle of the Plasmodium lophurae parasite. Since the results of the two series very closely approximated each other, only the counts made on one of them are herein presented. The results are shown in Table 7 for the duck-duck passage, Table 8 for the duck-turkey passage, Table 9 for the 1st turkey-turkey passage, and Table 10 for the 2nd turkey-turkey passage.

Table 7

The Distribution of Growth Stages in Passage Ducks Nos. 54 and 76 Observed Throughout a Period of 4 to 8 Days of Infection at 3-Hour Intervals
(+ Sign Indicates Less Than 1 Count in 100 Cells Counted)

Day of Infection	Time of Count	Duck No. 54				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	9AM	43	53	4	4	+
	12	41	56	2	1	+
	3PM	28	63	8	1	+
	6	29	71	+	+	+
	9	24	72	4	+	+
5	12	29	60	8	3	+
	3AM	25	61	11	3	+
	6	19	70	9	2	+
	9	33	56	9	2	+
	12	37	56	4	3	+
	3PM	41	47	8	4	+
	6	51	31	12	5	1
	9	55	37	4	3	1
6	12	59	38	3	+	+
	3AM	47	47	5	1	+
	6	47	46	6	1	+
	9	43	51	6	+	+
	12	33	52	13	2	+
	3PM	26	69	4	1	+
	6	14	72	13	1	+
	9	18	69	10	+	2
7	12	19	68	10	3	1
	3AM	25	59	10	5	1
	6	29	48	12	9	2
	9	44	25	20	11	+
	12	59	20	11	8	2
	3PM	51	36	9	1	3
	6	42	45	4	8	1
8	9	42	48	6	3	1

Table 7 (Continued)

Day of Infection	Time of Count	Duck No. 67				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	9AM	46	54	+	+	+
	12	49	47	4	+	+
	3PM	41	57	1	1	+
	6	47	50	2	1	+
	9	35	61	4	+	+
5	12	27	67	6	+	+
	3AM	23	76	1	+	+
	6	19	79	2	+	+
	9	21	65	12	2	+
	12	31	58	9	2	+
	3PM	40	35	16	9	+
	6	37	51	8	3	1
	9	55	34	5	5	1
6	12	74	20	6	+	+
	3AM	61	33	5	+	1
	6	37	55	1	4	3
	9	51	45	3	1	+
	12	47	46	6	1	+
	3PM	29	62	8	1	+
	6	13	76	11	+	+
	9	18	72	10	+	+
7	12	23	63	12	2	+
	3AM	24	60	13	3	+
	6	26	55	16	3	+
	9	27	41	27	5	+
	12	39	36	19	3	3
	3PM	29	52	18	1	+
	6	19	49	24	7	1
8	9	18	51	18	9	4

Table 8

The Distribution of Growth Stages in the Duck-Turkey Passage Observed in Turkeys Nos. 71, 72, 73, and 74 Throughout a Period of 4 to 7 Days at 3-Hour Intervals (+ Sign Indicates Less Than 1 Count in 100 Cells Counted)

Day of Infection	Time of Count	Turkey No. 71				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	53	42	2	2	1
	3PM	56	39	3	1	1
	6	54	37	8	1	+
	9	26	65	5	2	+
4	12	20	64	9	5	2
	3AM	23	66	9	1	1
	6	25	63	10	1	1
	9	27	64	3	6	+
	12	36	49	8	5	2
	3PM	38	43	10	7	2
	6	50	26	7	14	3
	9	31	49	4	9	7
5	12	62	32	2	2	2
	3AM	63	25	4	8	+
	6	53	36	3	5	3
	9	43	42	8	5	2
	12	32	59	4	4	1
	3PM	26	64	7	2	1
	6	22	55	14	7	2
	9	29	60	6	4	1
6	12	25	56	9	9	1
	3AM	38	54	5	2	1
	6	24	56	8	10	2
	9	34	56	4	3	3
	12	51	31	5	10	3
	3PM	45	30	14	9	2
	6	46	31	7	13	3
	9	49	40	4	6	1
7	12	45	35	3	13	4
	3AM	40	56	1	2	1
	6	21	66	10	2	1
	9	16	71	8	3	1
	12	24	66	6	2	2

Table 8 (Continued)

Day of Infection	Time of Count	Turkey No. 72				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	28	70	2	+	+
	3PM	8	75	16	+	1
	6	7	76	15	2	+
	9	6	88	6	+	+
4	12	10	61	20	5	4
	3AM	26	59	11	2	2
	6	46	28	8	16	2
	9	62	16	3	11	8
	12	60	21	7	9	3
	3PM	59	32	2	4	3
	6	50	45	2	1	2
	9	29	69	1	1	+
5	12	34	59	6	1	+
	3AM	25	69	4	2	+
	6	19	77	4	+	+
	9	7	82	8	1	2
	12	5	84	8	3	1
	3PM	11	64	16	8	1
	6	14	60	15	9	2
	9	34	43	10	10	3
6	12	47	32	4	13	4
	3AM	54	30	6	5	5
	6	58	27	2	11	2
	9	64	32	+	3	1
	12	52	42	2	2	2
	3PM	49	49	1	1	+
	6	35	62	3	+	+
	9	33	66	1	+	+
7	12	26	65	9	+	+
	3AM	17	73	6	4	+
	6	19	65	12	4	+
	9	25	63	10	2	+
	12	27	47	7	17	2

Table 8 (Continued)

Day of Infection	Time of Count	Turkey No. 73				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	54	42	4	+	+
	3PM	50	43	3	1	3
	6	52	43	1	2	2
	9	32	60	6	+	2
4	12	27	56	12	4	1
	3AM	23	71	5	1	+
	6	29	61	9	1	+
	9	25	69	5	1	+
	12	19	64	10	6	1
	3PM	23	54	10	9	4
	6	20	51	12	12	5
	9	45	36	12	4	3
5	12	52	35	3	5	5
	3AM	56	24	11	3	6
	6	47	39	8	6	+
	9	41	44	4	9	2
	12	26	63	7	3	1
	3PM	29	67	2	2	+
	6	23	58	11	4	4
	9	25	62	12	1	+
6	12	16	58	17	9	+
	3AM	13	76	7	4	+
	6	15	63	16	6	+
	9	22	57	16	5	+
	12	36	40	5	14	5
	3PM	43	35	5	16	1
	6	59	21	1	15	5
	9	55	30	4	8	3
7	12	50	40	5	2	3
	3AM	44	38	4	11	3
	6	30	64	5	1	+
	9	24	70	3	3	+
	12	20	71	2	6	1

Table 8 (Continued)

Day of Infection	Time of Count	Turkey No. 74				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	59	32	3	2	4
	3PM	58	32	4	2	4
	6	47	51	1	1	+
	9	44	49	7	+	+
4	12	32	64	3	2	1
	3AM	20	75	5	+	+
	6	24	66	5	4	1
	9	25	58	5	1	1
	12	24	68	7	+	1
	3PM	19	62	15	4	+
	6	24	60	10	6	+
	9	48	39	7	5	1
5	12	37	39	5	12	7
	3AM	68	20	5	5	2
	6	63	24	6	5	2
	9	59	29	6	3	3
	12	50	43	3	4	+
	3PM	48	47	2	2	1
	6	33	52	13	2	+
	9	22	65	6	5	2
6	12	20	71	6	3	+
	3AM	20	73	7	+	+
	6	22	61	10	6	1
	9	24	68	3	4	1
	12	37	52	3	8	+
	3PM	34	36	6	21	5
	6	48	29	6	14	3
	9	43	35	5	11	6
7	12	39	29	10	14	8
	3AM	35	54	3	7	1
	6	28	52	12	7	1
	9	25	62	8	4	1
	12	23	60	6	10	1

Table 9

The Distribution of Growth Stages in the 1st Turkey-Turkey Passage Observed in Turkeys Nos. 83, 84, 85, and 86 Throughout a Period of 4 to 7 Days at 3-Hour Intervals (+ Sign Indicates Less Than 1 Count in 100 Cells Counted)

Day of Infection	Time of Count	Turkey No. 83				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	48	44	3	2	3
	3PM	58	36	3	3	+
	6	31	64	3	+	2
	9	41	49	7	2	1
4	12	27	59	11	2	2
	3AM	34	61	3	2	+
	6	30	61	6	1	2
	9	30	54	9	4	3
	12	23	62	7	7	1
	3PM	25	65	7	3	+
	6	47	41	7	4	1
	9	42	49	6	3	+
5	12	46	44	6	3	1
	3AM	43	46	7	4	+
	6	33	52	12	3	+
	9	45	42	4	8	+
	12	18	65	8	9	+
	3PM	36	49	10	3	2
	6	23	63	7	6	1
	9	18	57	14	10	1
6	12	16	64	12	7	1
	3AM	20	40	17	19	4
	6	22	48	13	16	1
	9	25	44	13	17	1
	12	23	38	17	21	1
	3PM	19	43	20	16	2
	6	32	35	9	22	2
	9	38	35	15	12	+
7	12	46	24	6	2	2
	3AM	Death	Death	Death	Death	Death

Table 9 (Continued)

Day of Infection	Time of Count	Turkey No. 84				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	52	43	+	3	2
	3PM	46	46	3	3	2
	6	52	43	3	+	2
	9	27	64	5	4	+
4	12	25	61	9	4	1
	3AM	31	60	6	2	1
	6	25	69	5	+	1
	9	22	70	5	2	1
	12	26	66	5	1	2
	3PM	35	56	6	3	+
	6	42	49	6	3	+
	9	49	46	3	2	+
5	12	36	58	4	1	1
	3AM	49	41	5	3	2
	6	30	65	4	+	1
	9	50	45	5	+	+
	12	30	66	4	+	+
	3PM	29	54	8	8	1
	6	30	58	7	5	+
	9	15	67	8	10	+
6	12	21	52	9	18	+
	3AM	25	52	10	11	2
	6	26	55	12	6	1
	9	27	51	9	13	+
	12	25	53	11	9	2
	3PM	28	35	16	19	2
	6	31	41	11	15	2
	9	42	39	6	11	2
7	12	31	39	15	15	+
	3AM	30	42	14	14	+
	6	30	44	13	13	+
	9	26	55	14	5	+
	12	32	56	14	8	+

Table 9 (Continued)

Day of Infection	Time of Count	Turkey No. 85				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	49	47	+	4	+
	3PM	32	57	9	+	2
	6	36	54	4	5	1
	9	45	47	4	2	2
4	12	36	54	6	3	1
	3AM	35	52	8	3	2
	6	32	62	1	4	1
	9	39	47	13	1	+
	12	36	54	5	3	2
	3PM	36	57	6	1	+
	6	39	52	5	4	+
	9	37	52	4	7	+
5	12	40	48	10	2	+
	3AM	46	40	10	4	+
	6	37	52	7	3	1
	9	43	48	6	2	1
	12	29	66	2	3	+
	3PM	24	66	7	1	2
	6	18	72	4	4	2
	9	24	57	11	6	2
6	12	20	63	10	7	+
	3AM	22	45	22	9	2
	6	24	62	5	8	1
	9	21	55	8	14	2
	12	25	60	8	3	4
	3PM	21	54	11	10	4
	6	35	37	15	13	+
	9	28	48	18	6	+
7	12	22	41	23	12	2
	3AM	20	47	20	13	+
	6	18	48	19	15	+
	9	26	44	18	12	+
	12	25	45	15	15	+

Table 9 (Continued)

Day of Infection	Time of Count	Turkey No. 86				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	44	50	3	2	1
	3PM	41	50	6	2	1
	6	40	52	4	3	1
	9	35	54	9	2	+
4	12	29	61	6	3	1
	3AM	34	59	6	+	1
	6	34	53	5	5	3
	9	38	56	4	2	+
	12	30	61	8	1	+
	3PM	36	55	6	3	+
	6	38	52	3	5	2
	9	39	53	5	2	1
5	12	41	48	7	3	1
	3AM	40	52	5	3	+
	6	33	61	4	2	+
	9	46	51	3	+	+
	12	32	61	7	+	+
	3PM	36	52	7	3	2
	6	17	65	11	4	3
	9	16	66	10	7	1
6	12	10	69	15	8	+
	3AM	20	60	11	8	1
	6	22	58	8	12	+
	9	32	57	10	1	+
	12	31	59	7	3	1
	3PM	28	43	17	13	1
	6	29	39	13	17	2
	9	25	41	14	15	5
7	12	20	42	19	19	+
	3AM	12	50	18	20	+
	6	23	39	16	22	+
	9	Death	Death	Death	Death	Death

Table 10

The Distribution of Growth Stages in the 2nd Turkey-Turkey Passage Observed in Turkeys Nos. 87, 88, and 89 Throughout a Period of 4 to 7 Days at 3-Hour Intervals
(+ Sign Indicates Less Than 1 Count in 100 Cells Counted)

Day of Infection	Time of Count	Turkey No. 87				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	32	66	1	1	+
	3PM	28	62	6	3	1
	6	26	38	11	5	+
	9	24	65	7	3	1
4	12	24	63	7	4	2
	3AM	20	70	6	4	+
	6	22	74	1	3	+
	9	26	64	6	4	+
	12	29	61	6	3	1
	3PM	37	50	7	3	3
	6	27	65	7	+	1
	9	30	53	13	4	+
5	12	27	54	11	7	1
	3AM	20	64	8	8	+
	6	21	63	13	2	1
	9	28	43	14	15	+
	12	16	56	17	11	+
	3PM	13	55	21	10	1
	6	17	43	11	29	+
	9	23	49	10	18	+
6	12	26	44	19	11	+
	3AM	23	41	7	29	+
	6	48	31	5	16	+
	9	35	43	5	17	+
	12	27	62	5	5	1
	3PM	32	51	4	13	+
	6	20	74	3	3	+
	9	14	68	10	8	+
7	12	16	74	6	4	+
	3AM	10	81	6	3	+
	6	17	73	6	4	+
	9	12	82	6	+	+
	12	14	80	4	2	+

Table 10 (Continued)

Day of Infection	Time of Count	Turkey No. 88				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	25	71	2	2	+
	3PM	26	65	7	2	+
	6	20	61	11	7	1
	9	20	72	6	1	1
4	12	24	59	12	5	+
	3AM	18	70	10	+	2
	6	37	47	8	7	1
	9	26	60	8	5	1
	12	31	62	6	1	+
	3PM	40	43	9	8	+
	6	30	56	9	5	+
	9	35	52	12	1	+
5	12	27	66	3	4	+
	3AM	28	62	6	4	+
	6	20	66	12	2	+
	9	25	57	8	9	1
	12	23	34	22	21	+
	3PM	27	51	9	13	+
	6	24	52	10	14	+
	9	27	43	14	16	+
6	12	28	40	7	24	1
	3AM	28	28	13	31	+
	6	23	48	11	16	2
	9	Death	Death	Death	Death	Death

Table 10 (Continued)

Day of Infection	Time of Count	Turkey No. 89				
		Rings	Old Trophs.	2 - 5 Nuclei	6 or More Nuclei	Segmenters
	12	36	60	4	‡	‡
	3PM	24	61	8	5	2
	6	22	69	9	‡	‡
	9	17	75	8	‡	‡
4	12	15	74	9	2	‡
	3AM	24	69	2	5	‡
	6	29	61	8	‡	2
	9	27	60	8	5	‡
	12	35	59	2	3	1
	3PM	32	49	10	8	1
	6	35	60	3	2	‡
	9	26	59	3	11	1
5	12	40	55	4	1	‡
	3AM	27	59	9	4	1
	6	21	71	6	2	‡
	9	25	54	14	7	‡
	12	27	47	13	11	2
	3PM	18	65	11	6	‡
	6	28	51	7	13	1
	9	27	49	9	15	‡
6	12	28	51	14	7	‡
	3AM	35	41	9	15	‡
	6	28	44	9	18	1
	9	25	44	9	21	1
	12	29	56	4	9	2
	3PM	22	56	10	11	1
	6	21	53	12	13	1
	9	14	56	16	13	1
7	12	21	52	6	21	‡
	3AM	10	64	10	16	‡
	6	16	62	8	14	‡

In the experiment on the effects of the host on the asexual cycle of the lophurae parasite, as shown in Tables 7 to 10, two ducks were used for the duck passage, four turkeys for the duck-turkey passage, four for the 1st turkey-turkey passage, and three for the 2nd turkey-turkey passage. It may be noted in the tables that the data for individual birds on the stages of the asexual cycle in each passage approximate each other very closely. This being so, only 2 or 3 birds from each passage or group are presented in Figures 1, 2, and 3 for the duck cycle, duck-turkey cycle, and 1st turkey-turkey cycle, respectively. The data for the 2nd turkey-turkey cycle were not shown graphically because the data for this passage were similar to those for the 1st turkey-turkey passage. Curves for the ring stages were used in the graphs to determine the length of the asexual cycle. Data on counts for forms with 2 to 5 nuclei, 6 or more nuclei, and segmenters proved to be of little significance in this particular case.

That the differences in ring numbers are real and not apparent due to probable error in counting is shown by the fact that each figure represents a trend which persisted in a series of successive blood smears.

Synchronicity. As shown in Tables 7 to 10 and Figures 1 to 3, Plasmodium lophurae in ducks as well as in turkeys exhibited a low degree of synchronicity, that is, all growth stages were present at different times of the day and night

Figure 1. The distribution of ring stages, showing normal 36-hour asexual cycle in ducks Nos. 54 and 67 of the duck passage, observed over a period of 5 to 8 days at 3-hour intervals.

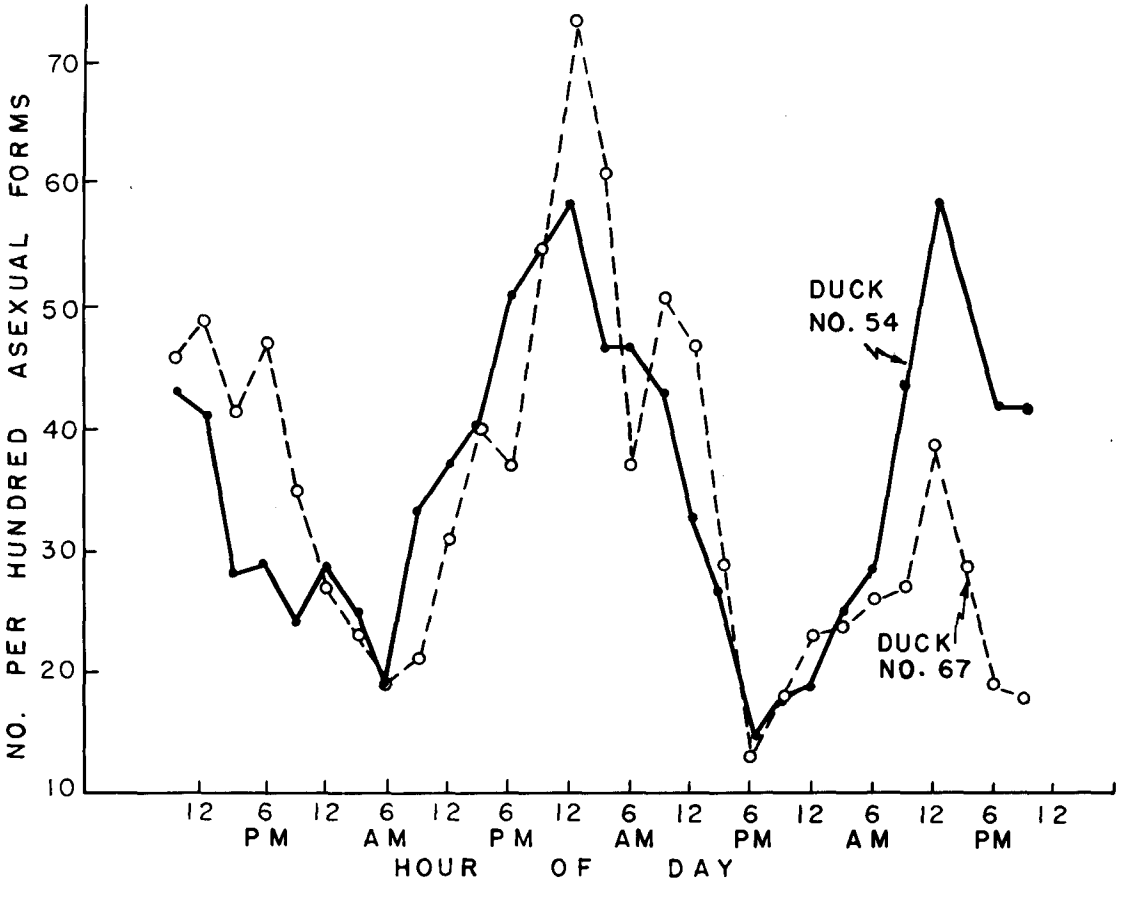

NO. PER HUNDRED ASEXUAL FORMS
70
60
50
40
30
20
10
DUCK NO. 54
DUCK NO. 67
12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12
PM AM PM AM PM AM PM
HOUR OF DAY

Figure 2. The distribution of ring stages, showing a predominantly 39-hour asexual cycle in turkeys No.s 71 and 72 of the duck-turkey passage, observed over a period of 4 to 7 days at 3-hour intervals.

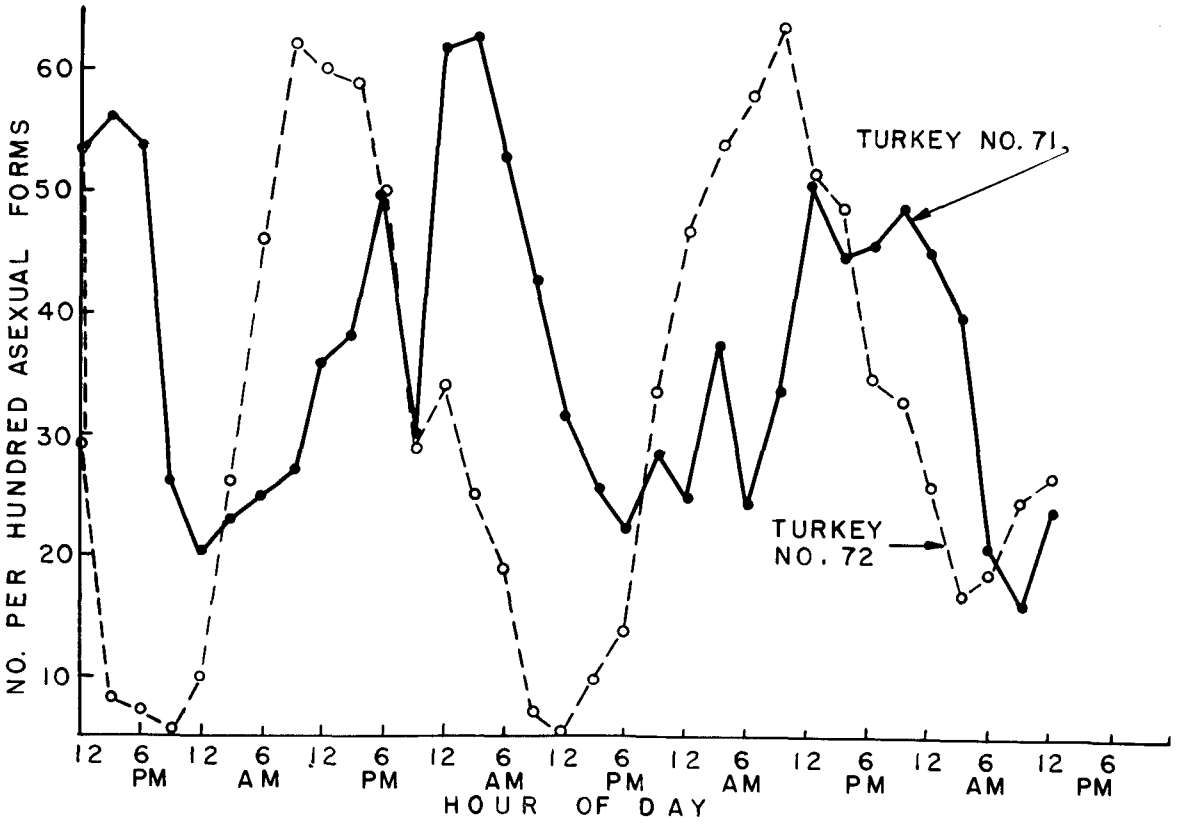
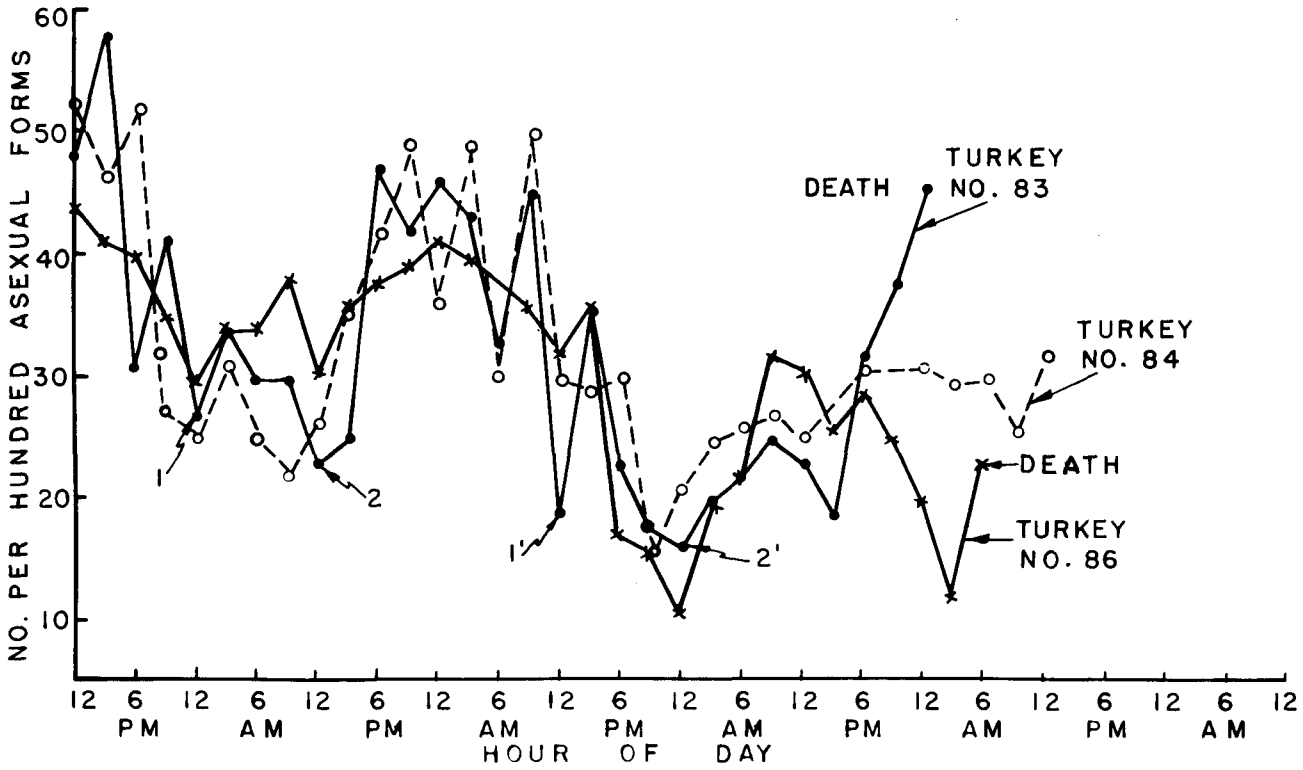

NO. PER HUNDRED ASEXUAL FORMS
60
50
40
30
20
10
TURKEY NO. 71
TURKEY NO. 72
12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6
PM AM PM AM PM AM PM AM PM
HOUR OF DAY

Figure 3. The distribution of ring stages in turkeys Nos. 83, 84, and 86 of the first turkey-turkey passage observed over a period of 4 to 7 days at 3-hour intervals, wherein turkey No. 83 presents 2 distinct broods with 36-hour asexual cycle (1-1'; 2-2'); turkey No. 84 shows a normal 36-hour cycle; and turkey No. 86 shows a reduction in cycle from 36 to 27 hours..


NO. PER HUNDRED ASEXUAL FORMS
60
50
40
30
20
10
12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12 6 12
PM AM PM AM PM AM PM AM PM AM
HOUR OF DAY
DEATH
TURKEY NO. 83
TURKEY NO. 84
DEATH
TURKEY NO. 86
1
2
1'
2'

throughout all the periods studied. Mature segmenters were found at each observation, although they were less than 1 per cent most of the time, and their number fluctuated very little.

Other species of bird malaria parasites show similarly low synchronicity, although no species yet described is altogether asynchronous (Hewitt, 22).

Periodicity and length of asexual cycle. Certain growth stages were more predominant at some times than at other times, as shown in the foregoing tables and figures, in spite of the fact that segmentation of the lophurae parasite was not restricted to a definite time interval. The time interval between peaks of any particular stage, particularly with reference to rings and ameboids, was in the majority of instances nearly 36 hours in the duck (Table 7, figure 1), 39 hours in the turkeys inoculated with parasitized duck blood (Table 8, Figure 2), and 36 hours in the turkey-turkey passages (Tables 9 and 10, Figure 3). Furthermore, the peaks in rings, and to a lesser degree in old trophozoites, follow one another in fairly consistent fashion, indicating regular periods of growth.

A close examination of the duck cycle as shown in Table 7 and Figure 1, reveals variations in the cycle occurring up to 45 hours. In the case of duck No. 67, the old trophozoite forms showed apparent lack of synchronicity in the

cycle in that there were no distinct groupings of this form.

The stability of the lophurae strain used in this experiment is shown by the fact that, although synchronicity was low, the peaks and troughs of the ring stages were fairly distinct and in different birds recurred after the same intervals of time.

Most of the variation in the length of the asexual cycle of the parasite occurred in the duck-turkey passage. In this case the average change in asexual cycle was prolonged by 3 hours. The variations among the 4 birds in this passage (Table 8, Figure 2) was from 36 hours to as high as 48 hours.

When the passage of the lophurae parasite was among turkeys, as in the 1st and 2nd turkey-turkey inoculations (Tables 9 and 10 and Figure 3), the length of asexual cycle went back close to the characteristic 36 hours. Counts of the peaks as well as the troughs in both ring and old trophozoite stage showed this fact in the 4 birds used in the first and the 3 birds used in the second turkey-turkey passages. Examination of the data in the foregoing tables and figures show the length of asexual cycle to vary from 27 to 36 hours, but the predominating length of the cycle was at 36 hours. In some cases, as in turkey No. 83, two broods with 36-hour cycle may be traced (marked in Figure 3 as 1-1', and 2-2'). A similar case of having two broods in the ring stages can be traced in turkey No. 74 of the duck-turkey passage, ex-

cept that each brood had a 39-hour cycle (Table 8).

It is interesting to note the change in the length of asexual cycle in turkey No. 86 of the 1st turkey-turkey passage and No. 88 of the 2nd turkey-turkey passage, since these birds died of the infection on the 6th or 7th day. In these birds the length of asexual cycle prior to death was shortened to 27 hours. Turkey No. 86 represented this case as shown in the graph in Figure 3. In the case of turkey No. 83 of the 1st turkey-turkey passage (Table 9) this shortening of the cycle to 27 hours prior to death was shown in the counts of old trophozoite forms, while it did not show in the ring stages. It will also be noted that in all three birds, the length of asexual cycle previous to this shortening was 36 hours as shown in the tables and figures referred to above. The above facts are demonstrated in the tables by the counts of old trophozoite forms in turkey No. 83, and in the counts of ring stages in turkeys Nos. 86 and 88.

Turkeys Nos. 83 and 86 reached their peak of parasitemia on the 6th day, with 69 and 66 per cent, respectively. In the other turkey which succumbed, No. 88, the peak of parasitemia was reached on the 5th day at 47.5 per cent and it died on the 6th day. In this bird, the cycle was found to be 27 hours as shown by the count of raing stages only, since the old trophozoite forms lacked synchronicity.

In general, the turkey-turkey passages showed a pre-

dominatly 36-hour cycle, except for the last cycle preceding death, which was 27 hours.

Published data concerning the length of the asexual cycle of Plasmodium lophurae are those by Coggeshall in 1938 (10) and by Terzian in 1941 (43) in which chickens were used as experimental hosts. The former author believed the asexual cycle to be 24 hours in length, and the latter found it to be 48 hours. Hewitt in 1942 (22) working with ducks as host found that the asexual cycle of Plasmodium lophurae averaged about 36 hours in length under a 12 hours light and dark routine maintained in the animal quarters. Taliaferro and Taliaferro in 1950 (42) also reported that the parasite in the chickens tested showed a 36 hour cycle. All the above authors noted the low synchronicity of the parasite.

The only studies of host effects on Plasmodium are those of Hegner and West in 1940 (20, 21) on Plasmodium cathemerium. They found no change in the length of asexual cycle of 24 hours upon the change of host from canaries to ducks, while the transfer of the parasite either from duck or canaries to chicks made the length of asexual cycle approximately 48 hours. Why this is so, as the authors stated, is an interesting but unsolved problem.

Temperature changes during the course of infection

Rectal temperatures were taken at 6-hour intervals during the course of infection in a group of 3 27-day-old turkeys with Plasmodium lophurae. A control group of 3 turkeys were maintained. Daily parasite count of all infected individuals were made. The turkeys were kept in cages in the laboratory at room temperature and no attempts were made to regulate the room temperature.

The data obtained are presented in Table 11 for the control group, and Table 12 for the parasitized group. The temperatures were taken at 6 P. M., 12 P. M., 6 A. M. and 12 A. M. during the course of infection for 13 consecutive days. Table 13 shows the comparison of the daily mean temperatures of the parasitized turkeys and their unparasitized controls. Three types of infection were represented, relapsing in turkey No. 87, fatal in No. 88, and resistant in No. 89. The data in this table are graphically presented in Figure 4.

Temperature changes in the control unparasitized turkeys. As shown in Table 11, the normal temperature of turkey poults of about a month old seemed to be affected to some extent by excitement or by periods of rest and activity. The rectal temperature readings at 12 AM (noon) and at 6 PM were generally slightly higher than those of temperature readings taken at 12 PM (mid-night) and at 6 AM when the

Table 11

Rectal Temperatures in Degrees Fahrenheit of 3 Unparasitized Turkeys, Taken at 6-Hour Intervals on 13 Successive Days

Time of Day	No. of Turkey	Days After Infection						
		1	2	3	4	5	6	7
6PM	90	107.6	107.4	107.2	107.8	106.6	106.6	106.8
	91	107.6	107.2	107.2	107.6	106.4	106.8	106.8
	92	107.6	107.2	107.2	107.0	107.4	106.8	106.4
	Mean	107.60	107.26	107.20	107.46	106.80	106.73	106.66
		±0.00	±0.01	±0.00	±0.12	±0.23	±0.01	±0.05
12	90	106.8	106.4	106.2	106.2	106.2	106.0	105.8
	91	106.2	106.6	106.2	106.8	105.2	105.2	105.6
	92	107.0	106.4	105.8	106.0	106.2	106.0	105.0
	Mean	106.66	106.46	106.06	106.33	105.86	105.73	105.46
		±0.18	±0.01	±0.05	±0.17	±0.33	±0.21	±0.17
6AM	90	106.8	106.4	106.6	106.8	106.2	106.2	106.4
	91	106.8	106.6	106.2	106.4	106.4	106.2	106.2
	92	106.0	106.0	105.6	106.0	105.4	105.4	105.4
	Mean	106.53	106.33	106.13	106.40	106.00	105.93	106.00
		±0.21	±0.09	±0.25	±0.21	±0.28	±0.21	±0.28
12	90	107.0	106.6	107.2	107.2	106.8	107.2	107.0
	91	107.0	107.0	106.8	106.8	106.6	106.8	106.4
	92	107.2	106.8	106.8	106.4	106.2	106.6	106.4
	Mean	107.06	106.80	106.93	106.80	106.53	106.86	106.60
		±0.01	±0.04	±0.05	±0.16	±0.09	±0.09	±0.12
	Daily Mean	106.96	106.71	106.58	106.75	106.30	106.31	106.18

Table 11 (Continued)

Time of Day	No. of Turkey	Days After Infection 8	9	10	11	12	13
	90	107.2	107.0	106.8	107.2	107.0	107.0
	91	107.2	106.8	106.8	107.2	107.0	107.2
6PM	92	107.0	106.6	106.6	106.8	107.0	107.0
	Mean	107.13	106.80	106.73	107.06	107.00	107.06
		±0.01	±0.04	±0.01	±0.05	±0.00	±0.01
	90	106.2	106.2	105.8	106.0	106.4	105.8
	91	105.8	105.8	106.0	106.2	106.2	106.0
12	92	105.0	105.2	105.4	105.8	105.2	105.0
	Mean	105.66	105.73	105.73	106.00	105.93	105.60
		±0.37	±0.25	±0.09	±0.04	±0.41	±0.28
	90	106.2	106.4	106.4	106.6	106.2	106.0
	91	106.3	106.4	106.2	106.4	105.8	105.8
6AM	92	105.4	105.6	105.6	105.8	105.4	105.2
	Mean	105.93	106.13	106.06	106.26	105.80	105.66
		±0.21	±0.21	±0.17	±0.87	±0.16	±0.17
	90	107.0	106.8	107.0	107.0	107.0	107.0
	91	106.6	106.4	106.6	107.0	107.0	106.8
12	92	106.2	106.2	106.2	106.0	106.0	106.2
	Mean	106.60	106.46	106.93	106.66	106.66	106.66
		±0.04	±0.09	±0.16	±0.38	±0.38	±0.01
	Daily Mean	106.33	106.28	106.36	106.49	106.35	106.24

Range, 156 Readings = 105.0 to 107.8°F

Mean = 106.45°F ±0.24

Table 12

Rectal Temperatures in Degrees Fahrenheit of 3 Parasitized Turkeys, Taken at 6-Hour Intervals on 13 Successive Days

Time of Day	No. of Turkey	Days After Infection						
		1	2	3	4	5	6	7
6PM	87	107.6	107.8	108.0	107.6	107.8	106.2	103.8
	88	107.6	107.6	107.6	107.8	108.2	105.2	
	89	107.4	107.6	107.4	107.4	107.2	106.0	104.0
	Mean	107.53	107.66	107.66	107.60	107.73	105.80	
		±0.12	±0.01	±0.30	±0.20	±0.50	±0.52	
12	87	108.2	106.8	106.8	106.8	107.0	103.4	102.8
	88	107.4	105.8	106.2	106.8	107.6	102.2	
	89	108.2	106.4	106.4	106.8	106.4	104.8	103.0
	Mean	107.93	106.33	106.46	106.80	107.00	103.46	
		±0.40	±0.25	±0.09	±0.00	±0.36	±1.30	
6AM	87	107.2	106.6	106.6	107.2	106.0	102.4	102.4
	88	107.2	106.6	106.6	106.8	106.6	101.4	
	89	107.2	106.6	106.6	106.8	105.4	104.4	104.2
	Mean	107.20	106.60	106.60	106.93	106.80	102.73	
		±0.00	±0.00	±0.00	±0.05	±0.36	±1.58	
12	87	107.2	106.6	106.8	107.4	105.6	102.4	106.2
	88	106.8	106.6	107.0	107.8	106.6	Death	
	89	107.4	106.8	106.6	107.0	106.4	103.6	106.2
	Mean	107.13	106.73	107.13	107.40	106.20		
		±0.09	±0.01	±0.04	±0.16	±0.34		
	Daily Ave.	107.45	106.68	106.96	107.18	106.93		

Table 12 (Continued)

Time of Day	No. of Turkey	Days After Infection					
		8	9	10	11	12	13
6PM	87	106.6	107.0	106.4	105.4	105.8	106.4
	88						
	89	106.8	106.4	106.6	106.4	106.4	107.2
	Mean						
12	87	104.0	104.8	105.2	104.4	103.8	105.2
	88						
	89	105.8	105.6	105.6	106.0	106.0	106.0
	Mean						
6AM	87	104.0	104.8	104.4	104.6	103.8	105.0
	88						
	89	105.0	104.8	105.4	106.2	105.8	105.6
	Mean						
12	87	106.0	106.0	104.8	105.4	105.6	106.2
	88						
	89	105.8	106.2	106.0	106.2	106.4	106.4
	Mean						

Range (1 - 5 days) = 5.4 - 8.2°F

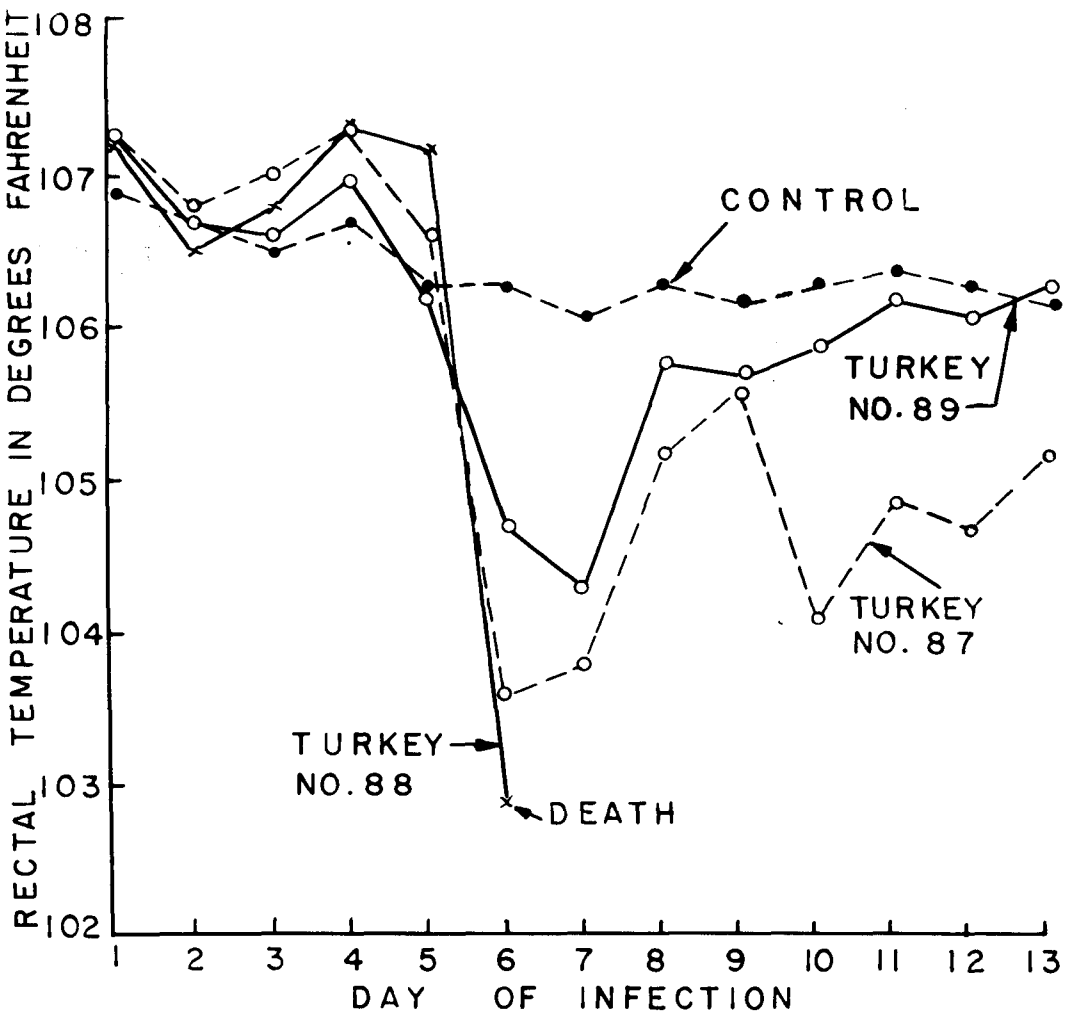
Mean (1 - 5 days) = 107.04°F ±0.28

Table 13

Relationship Between Parasite Number and Changes in Temperature During the Course of Infection Among 3 Classes of Infection in Turkeys, That is, Relapsing in No. 87, Fatal in No. 88, and Resistant in No. 89; and Comparison of the Daily Mean Rectal Temperature of Parasitized Turkeys and Their Unparasitized Controls

Day After Infection	Per Cent Parasitized Cells			Mean Daily Rectal Temperature in °F			
	Turkey Nos.			Turkey Nos.			
	87	88	89	87	88	89	Control
1	0.90	0.65	0.60	107.30	107.25	107.30	106.96
2	7.40	7.40	6.00	106.80	106.50	106.72	106.71
3	27.50	20.33	25.00	107.05	106.85	106.60	106.58
4	56.00	52.50	44.00	107.30	107.30	107.00	106.75
5	72.50	71.00	47.50	106.60	107.25	106.25	106.30
6	49.00	56.00 (Death)	36.00	103.60	102.93 (Death)	104.70	106.31
7	18.50		2.10	103.80		104.35	106.18
8	39.00		0.20	105.20		105.85	106.33
9	51.00		0	105.65		105.75	106.28
10	64.50			104.16		105.90	106.36
11	30.00		0	104.95		106.20	106.49
12	8.50		0	104.75		106.15	106.35
13	6.50		0	105.20		106.30	106.24

Figure 4. Comparison of the daily mean rectal temperature, taken at 6-hour intervals during the course of infection for 13 days, of the parasitized turkeys and their unparasitized controls. Types of infection presented: relapsing in 87, fatal in 88, and resistant in 89.


RECTAL TEMPERATURE IN DEGREES FAHRENHEIT
108
107
106
105
104
103
102
1
2
3
4
5
6
7
8
9
10
11
12
13
DAY OF INFECTION
CONTROL
TURKEY NO. 89
TURKEY NO. 87
TURKEY NO. 88
DEATH

birds were generally quiet and at rest. The average differences in these temperatures, however, were never more than 1.4°F.

The rectal temperature of unparasitized turkeys ranged from 105.00°F to 107.8°F, averaging 106.45°F ±0.24. Some fluctuation occurred from day to day in individual birds. The daily fluctuation for the group, however, was less than 1°F (see Figure 4).

Temperature changes during the course of infection with Plasmodium lophurae in turkeys; relationships between parasitemia and change in temperature. In general, in all the three types of infection (relapsing in No. 87, fatal in 88, and resistant in 89), there was little difference between the temperatures of parasitized turkeys for the 1st to the 4th or 5th days after infection and those in the unparasitized control turkeys (see Tables 13 and Figure 4), when fluctuations are considered. A test of significance was made of these differences in temperatures from the 1st to the 6th day, and only on the 6th day were the difference significant in all the 4 counts during the day, that is, at 6 PM, 12 PM, 6 AM and 12 AM. In other instances only 1 or 2 of the 4 daily temperature readings proved significant (the 12 PM and 12 AM temperatures on the 1st day, 6 PM temperature on the 2nd day, and the 12 PM temperature on the 5th day). The birds showed what appeared to be a temperature response

on the day after inoculation (see Table 12), but this difference was not significant.

On about the 3rd day after inoculation, the parasite number began to rise rapidly and the peak of parasitemia was reached on the 5th day. This relationship between parasite number and changes in temperature is shown in Figures 5 and 6.

As may be noted in Figures 5 and 6, the temperature during the 1st five days had a tendency to remain constant in turkey No. 87 (relapsing type), to rise in turkey No. 88 (fatal type), and to go down in turkey No. 89 (resistant type). Test of significance of these temperatures, however, showed that these differences in temperatures during the first 5 days were not significant from the variations that occurred in the control. A significant decline in temperature occurred after the 5th day, and in all cases, these abrupt drops in temperature started about a day just prior to the peak in the number of parasites (see Figures 5 and 6). The temperature dropped abruptly regardless of whether the parasite number continued to rise and the bird died, as in the case of turkey No. 88, or the parasite dropped precipitously with subsequent recovery as in the case of turkey No. 89 (Figure 6). In the case of a relapsing type of infection, such as turkey No. 87, (Figure 5), the same relationship seem to be indicated in the second peak of parasitemia as it

Figure 5. The relationship between parasite number and changes in rectal temperature during the course of primary infection and relapse in turkey No. 87. Observations were made at 6-hour intervals.

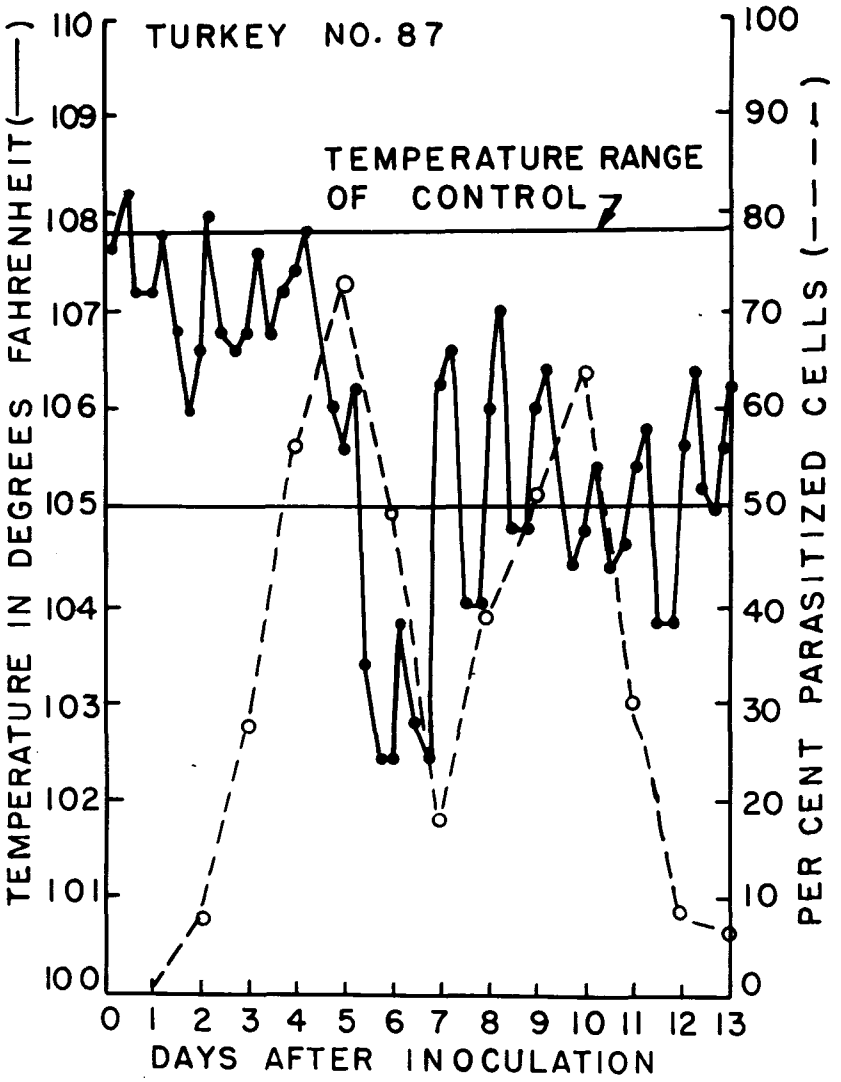
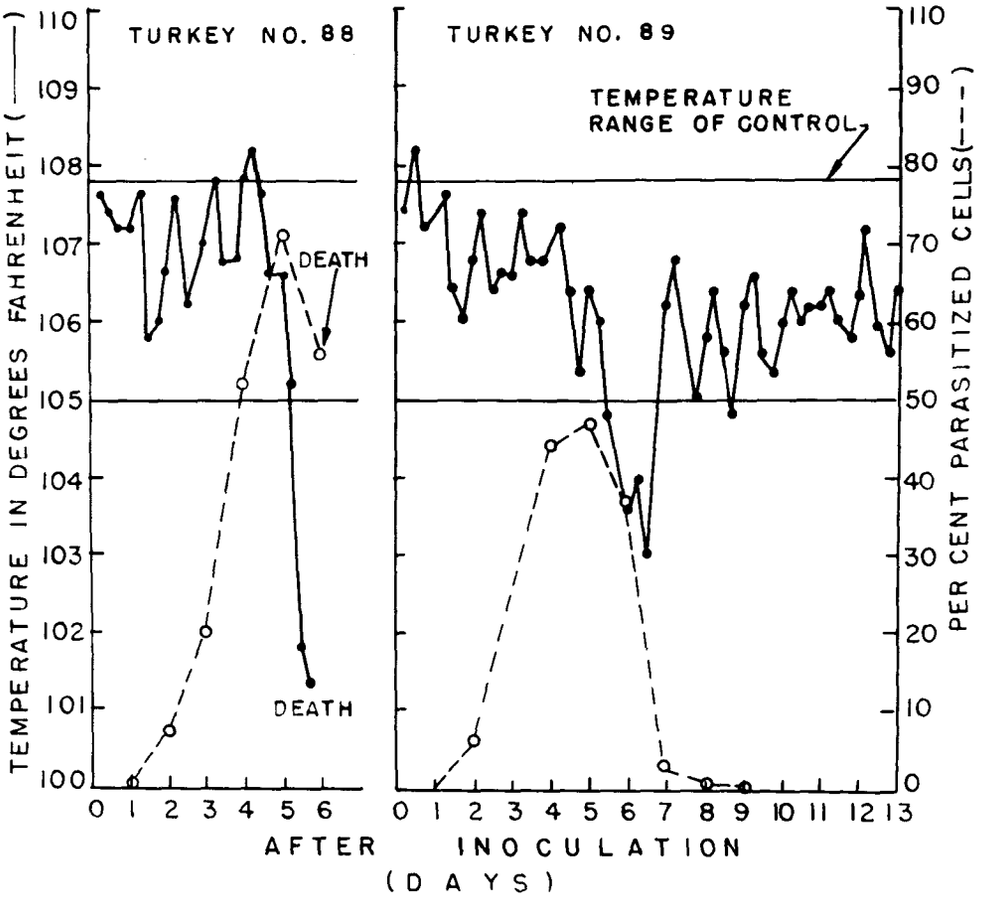

TURKEY NO. 87
TEMPERATURE RANGE OF CONTROL
TEMPERATURE IN DEGREES FAHRENHEIT (——)
PER CENT PARASITIZED CELLS (- - - -)
110
109
108
107
106
105
104
103
102
101
100
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
DAYS AFTER INOCULATION

Figure 6. The relationship between parasite number and and changes in rectal temperature during the course of infection in turkey No. 88 (fatal type) and turkey No. 89 (resistant type). Observations were made at 6-hour intervals.


TURKEY NO. 88
TURKEY NO. 89
TEMPERATURE RANGE OF CONTROL
DEATH
DEATH
TEMPERATURE IN DEGREES FAHRENHEIT (——)
PER CENT PARASITIZED CELLS(- - -)
110
109
108
107
106
105
104
103
102
101
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6
0 1 2 3 4 5 6 7 8 9 10 11 12 13
AFTER INOCULATION
(DAYS)

was on the first peak, only the fall of the temperature was not so marked. In the infections which terminated fatally, death occurred during the sharp drops in temperature which followed the peaks.

The foregoing results obtained in the present study are in agreement with those obtained by Hewitt in 1942 (22) in his study of the parasite in ducks.

As shown in Figure 6, it should be noted that the temperatures for turkey No. 88, which died of the infection, were within normal range of the controls after inoculation up to the 4th day. Hewitt (22) in his studies of infected ducks which succumbed with Plasmodium lophurae infection, found that the temperatures were slightly above normal shortly after inoculation, and thereupon they dropped to normal levels for 2 or 3 days. Coincident with the sharp rise in parasite number, the temperature rose above the normal fluctuation of unparasitized controls (designated in Figures 5 and 6 by heavy horizontal lines), but started dropping abruptly just before the parasite peak was reached, and continued to drop below the normal level coincident with the sharp decline in parasitemia. The lowest temperature recorded before death occurred was 101.4°F, or about 3.6°F below the lowest temperature reached in the uninfected control birds and 5°F below the average temperature of parasitized turkeys.

Relationship between growth stages of Plasmodium lophurae in turkeys and temperature. Examination of the temperatures of turkeys Nos. 87, 88, and 89 (Table 12) and their respective counts of growth stages (Table 10) show that none of the temperature data can be correlated with an increase in any particular growth stage. This result is in agreement with the results obtained by Hewitt in 1942 (22) in his study of the parasite in ducks.

Merozoite Production, Survival and Death Rates of *Plasmodium lophurae* During Primary Infection and Relapse in Parasitized Turkeys

The present work is limited to a study of initial infection and relapse with special reference to merozoite production and survival and death rates of the parasites formed.

The infections were blood-induced in turkeys with parasitized turkey erythrocytes, the dosage being the same for all the birds used, that is, 2.5×10^8 parasitized cells per 100 grams body weight. All parasite counts were made on smears that were taken at intervals of from 3 to 6 hours during the course of infection and relapse.

The methods of determining the rate of reproduction from the merozoite mean by comparing the number of merozoites fromed with the actual increase and decrease in blood population of the parasites, were the same as those used by Taliaferro and Taliaferro in their experiments in 1944 (38),

1947 (40), 1949 (41), and 1950 (42).

To ascertain the length and synchronism of the asexual cycle, differential counts were made of parasites as was done in the previous experiments.

The merozoite mean per segmenter for a given interval (3 to 6 hours) for the duration of initial infection and relapse was obtained after tabulating the number of merozoites in each of 25 or more mature segmenters from ordinary thin dried blood films stained with Giemsa. Maturity of a segmenter was considered to have been reached when the divided nuclei had separated into merozoites.

Changes in the basic rate of reproduction were considered to take place when the mean number of merozoites per segmenter changed. The survival rate of parasites at a given segmentation equals the actual rate of increase for that segmentation as ascertained by parasite counts. This was obtained by dividing the rate of increase by the merozoite mean per segmenter. Death rates were obtained by subtracting the rate of increase from the merozoite mean per segmenter, that is, total number of parasites produced. The actual rate of increase for those merozoites which developed into gametocytes was not corrected for by Taliaferro and Taliaferro in similar studies of the parasite in chicks (42), because they were scarce until the later part of the infection. A similar procedure was followed in this experiment.

A total of 25 turkeys were infected for this study,

but only three of them recorded relapses, so that only the results of observation on these 3 birds are presented herein. These results are presented in Table 14 for turkey No. 41, Table 15 for turkey No. 73, and Table 16 for turkey No. 87. The data summarized in these tables are taken from daily counts made at 3 to 6 hour intervals. In the above mentioned table, the daily count for merozoite mean per segmenter represent the mean of 4 counts taken at 6-hour intervals. Graphical representation of the parasitemia and rate of reproduction of the parasite are shown in Figures 7, 8, and 9 for turkeys Nos. 41, 73, and 87, respectively.

Segmenters of any one asexual cycle were composed of a number of merozoites varying from 6 to 30, usually 10 to 16, with a merozoite mean throughout the course of infection varying in general from 11 to 16.

As shown in Tables 14, 15, and 16 the factor of increase in the parasitemia as computed daily was highest in the primary infection, and decreased thereafter. Figures 7, 8, and 9 show the average parasitemia, merozoite mean per segmenter, and over all parasite survival and death rates during three relapsing infections. It can be noted that the results obtained from the study in the 3 turkeys were very similar during the initial infection. In turkeys Nos. 73 and 87, the parasitemia rose suddenly to a peak on about the 5th day of infection, then dropped down abruptly. The

Table 14

Parasitemia, Reproductive Rates, Death and Survival of Parasites in Turkey No. 41

Day After Infection	Per Cent Parasitized Cells	Rate of Increase	Merozoite Mean Per Segmenter	Per Cent Rate of		No. of Merozoites Survival Per Segmenter
				Survival	Death	
1	1.8					
2	6.4	3.55	13.60	26.10	73.90	3.55
3	26.0	4.06	14.73	27.56	72.44	4.06
4	40.0	1.54	14.98	10.28	89.72	1.54
5	52.0	1.30	12.71	10.23	89.77	1.30
6	65.0	1.25	11.64	10.74	89.26	1.25
7	30.0	0.46	14.03	3.28	96.72	0.46
8	6.8	0.23	13.81	1.66	98.34	0.23
9	1.2	0.18	14.00	1.28	98.72	0.18
10	0.7					
11	0.6					
12	4.4	7.33	14.25	51.44	48.56	7.33
13	8.0	1.82	14.80	12.30	87.70	1.82
14	11.0	1.37	13.93	9.83	90.17	1.37
15	24.0	2.82	13.61	20.72	79.28	2.82
16	7.8	0.32	14.11	2.27	97.71	0.32
17	1.0	0.13	14.01	0.93	99.07	0.13

Table 15

Parasitemia, Reproductive Rates, Death and Survival of Parasites in Turkey No. 73

Day After Infection	Per Cent Parasitized Cells	Rate of Increase	Merozoite Mean Per Segmenter	Per Cent Rate of		No. Of Merozoites Survival Per Segmenter
				Survival	Death	
1	2.33					
2	9.00	3.86	14.60	26.43	73.57	3.86
3	28.50	3.11	14.94	20.82	79.18	3.11
4	37.00	1.30	13.73	9.47	90.53	1.30
5	60.00	1.62	11.63	1.39	98.61	1.62
6	30.5	0.51	12.54	4.07	95.93	0.51
7	12.0	0.40	14.54	2.75	97.25	0.40
8	1.7	0.14	14.70	0.95	99.05	0.14
9	0.25					
10	0.10					
11	0.03					
12	1.40					
13	13.33	9.52	14.60	65.20	34.80	9.52
14	52.00	3.90	14.82	26.31	73.69	3.90
15	83.00	1.60	15.51	10.31	89.69	1.60
16	76.50	0.92	15.35	5.60	94.40	0.92

Table 16

Parasitemia, Reproductive Rates, Death and Survival of Parasites in Turkey No. 87

Day After Infection	Per Cent Parasitized Cells	Rate of Increase	Merozoite Mean Per Segmenter	Per Cent Rate of		No. of Merozoites Survival Per Segmenter
				Survival	Death	
1	1.90					
2	7.40	3.89	15.46	25.16	74.84	3.89
3	27.50	3.72	14.88	25.00	75.00	3.72
4	56.50	2.05	13.01	15.76	84.24	2.05
5	73.00	1.30	11.73	11.08	88.92	1.30
6	49.00	0.67	14.09	4.75	95.25	0.67
7	18.50	0.38	14.79	2.57	97.43	0.38
8	39.00	2.11	15.03	14.04	85.96	2.11
9	51.00	1.31	14.79	8.86	91.14	1.31
10	64.50	1.26	11.78	10.70	89.30	1.26
11	30.00	0.46	13.05	3.52	96.48	0.46
12	8.50	0.28	14.95	1.87	98.13	0.28
13	3.50	0.41	14.80	2.36	97.64	0.41

Figure 7. Infection in turkey No. 41 as shown by merozoite mean per segmenter, parasitemia, and survival and death of parasites. Merozoite mean, solid line; parasitemia, dash line; over-all parasite survival, i. e., merozoite survival per segmenter, shaded area; over-all parasite death per segmenter, clear area under merozoite mean.

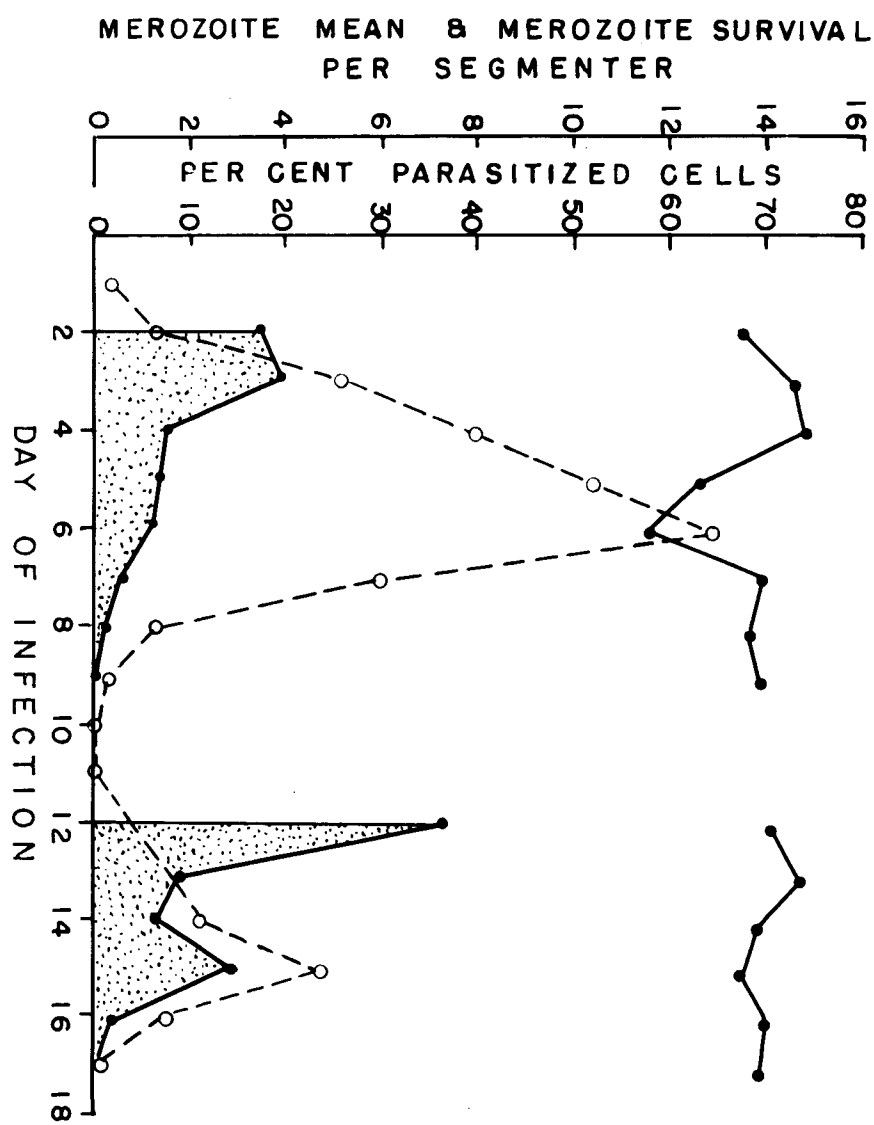

MEROZOITE MEAN & MEROZOITE SURVIVAL
PER SEGMENTER
0 2 4 6 8 10 12 14 16
PER CENT PARASITIZED CELLS
0 10 20 30 40 50 60 70 80
DAY OF INFECTION
2 4 6 8 10 12 14 16 18

Figure 8. Infection in turkey No. 73 as shown by merozoite mean per segmenter, parasitemia, and survival and death of parasites. Merozoite mean, solid line; parasitemia, dash line; over-all parasite survival, i. e., merozoite survival per segmenter, shaded area; over-all parasite death per segmenter, clear area under merozoite mean.

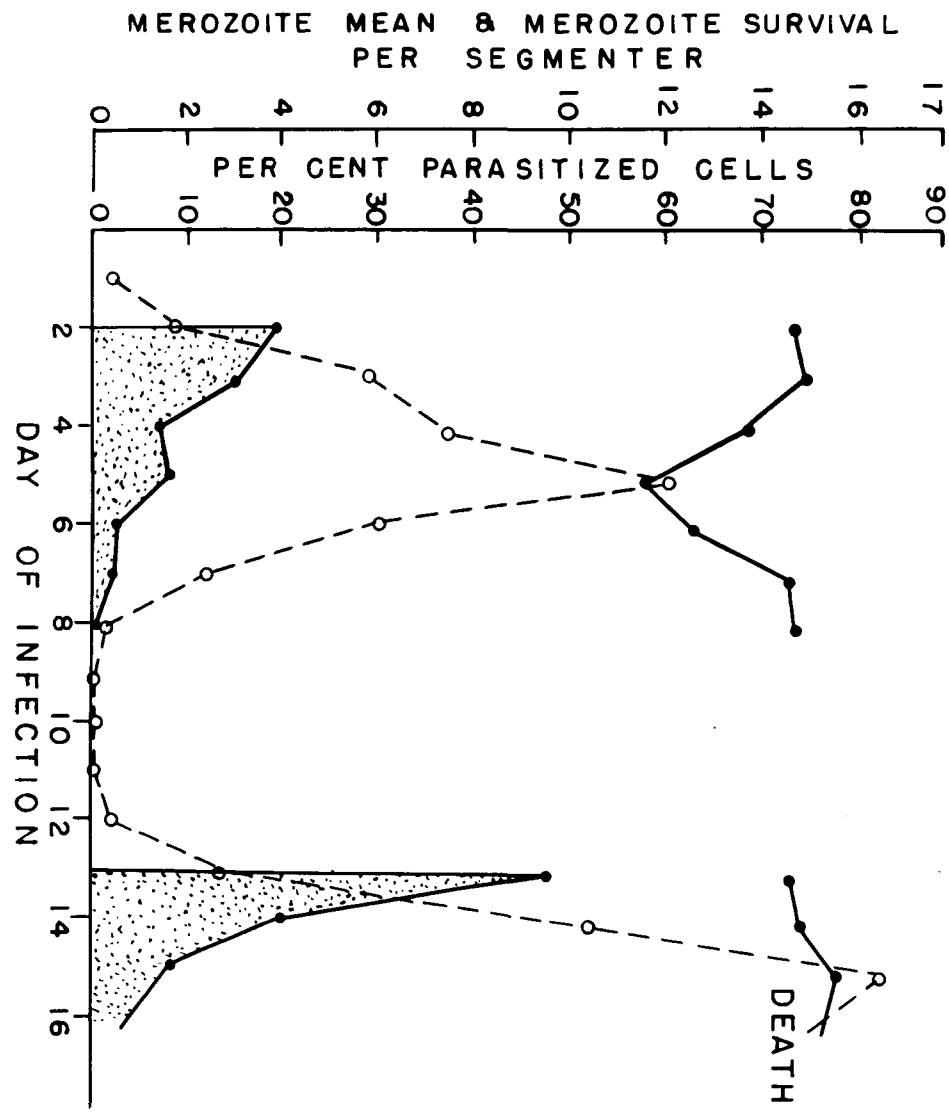
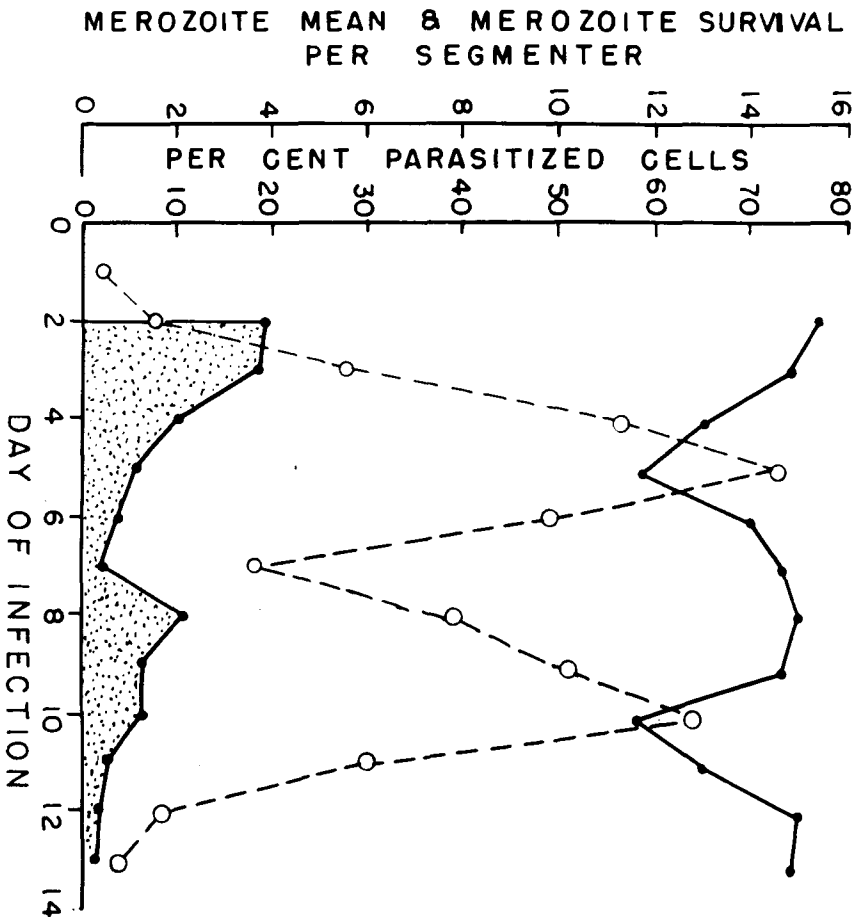

MEROZOITE MEAN & MEROZOITE SURVIVAL
PER SEGMENTER
0 2 4 6 8 10 12 14 16 17
PER CENT PARASITIZED CELLS
0 10 20 30 40 50 60 70 80 90
DAY OF INFECTION
2 4 6 8 10 12 14 16
R
DEATH

Figure 9. Infection in turkey No. 87 as shown by merozoite mean per segmenter, parasitemia, and survival and death of parasites. Merozoite mean, solid line; parasitemia, dash line; over-all parasite survival, i. e., merozoite survival per segmenter, shaded area; over-all parasite death per segmenter, clear area under merozoite mean.


MEROZOITE MEAN & MEROZOITE SURVIVAL PER SEGMENTER
0 2 4 6 8 10 12 14 16
PER CENT PARASITIZED CELLS
0 10 20 30 40 50 60 70 80
DAY OF INFECTION
0 2 4 6 8 10 12 14

merozoite mean per segmenter, however, started high as expected and decreased thereafter, reached its lowest level when the parasitemia was at its peak and then rose again to its normal level. Similar observations were made on turkey No. 41, which was inoculated with parasitized duck erythrocytes instead of turkey parasitized red blood cells as in the two previously named birds, with the exception that the peak of infection occurred a day later than in the two other birds. In all three birds the over-all survival rate of the parasite for the first day of infection was low (26.43% for turkey No. 73, 15.4% for turkey No. 87, and 13.60% for turkey No. 41).

Of the 13.6 merozoites produced in turkey No. 41, only 3.55 survived, only 3.86 of 14.60 survived in turkey No. 73, and only 3.88 of 15.46 survived in turkey No. 87. There was a gradual decline in survival rate during the succeeding segmentations of the initial infection until it became so that almost all of the parasites died, and at this stage the average of the number of segmenters that survived per asexual cycle was less than 1.

The above results obtained in the present study were similar to the result obtained by Taliaferro and Taliaferro in 1950 (42) in Plasmodium lophurae on chicks. In their studies of semi-acute infections, they reported that through the 3rd segmentation the parasites actually increased in

number although at a decreasing rate and thereafter they just maintained themselves or actually decreased. This point, as the authors stated, represented the initial peak of the infection in an immunological sense. It terminated the acute rise and initiated the parasite decline. So long as the parasite just maintained itself, an average of 1 merozoite per segmenter per asexual cycle survived. When they decreased in number, less than 1 parasite survived.

In the relapsing type of infection similar counts and observations were made as shown in Figures 7, 8, and 9. The three turkeys differed in the intensity of relapse. In turkey No. 87 the relapse occurred before the parasite counts became negative, while in the other birds the parasite counts dropped down to almost zero for 2 or 3 days before relapse occurred. The results of similar counts made of the initial infection on these 3 turkeys made during the course of relapse indicate that parasite survival was proportionally reflected in the intensity of the relapse. In turkey No. 73, which died from the relapse (Figure 8), 65.20% of parasites survived during the initial onset of relapse or 9.52 merozoites survived out of the 14.60 merozoites produced during this segmentation. For the initial infection 26.43% survived, or 3.86 of 14.6 merozoites produced survived (see Table 15). In the case of turkey No. 87 (Figure 9) where the relapse was less severe than the initial infection,

parasite survival was proportionally decreased (only 2.11 merozoite survived out of 15.03 in relapse or 14.04% compared to merozoite survival of 3.88 out of 15.46 merozoite produced or 25.16% for the initial infection), (See Table 16).

Contrary to expectations, in turkey No. 41 (Figure 7 and Table 14) the parasite survival in the relapse, which was mild, was higher than that in the initial infection. Survival rate was 81.44% on the onset of relapse compared to 26.10% in the initial infection. The fact that the parasite production remained at a fairly low level, being only 24.00%, so as to give a mild relapse, might explain this results. Such disparities are invariably encountered, and may also be due to inherent differences in individual turkeys.

The results for the relapsing infections are of further interest because the merozoite mean per segmenter was in general higher in rapidly fatal relapse infection than in the mild ones. The findings of this experiment agree with the findings of Taliaferro and Taliaferro (42) in their work with lophurae malaria in chicks. They stated that their results indicated that a high merozoite mean per segmenter can in general be correlated with high pathogenicity and thus, conversely, with low immunity. They concluded that the rate of reproduction of the parasite was maintained at a fairly constant rate except for a temporary inhibition of

about 20 to 30% at the crisis, whereas only one-fourth to one-half of the merozoite produced per segmenter survived during the acute rise and less than 1 merozoite per segmenter survived during the crisis. These changes were largely associated with the development of acquired immunity by the chicken.

The result of the present experiment are of particular interest in that they add direct evidence to the findings of the Taliaferros that much of the reduction of the number of merozoites produced by segmenters, which reaches its maximum near the crisis, is due to acquired immunity. In this study, evidence of acquired immunity appeared progressively against Plasmodium lophurae in turkeys. Taliaferro and Taliaferro (42), however, reported that acquired immunity developed progressively against Plasmodium gallinaceum, and suddenly at the peak of infection against Plasmodium lophurae, both in chickens.

Immunity Experiments

Experiments with duck plasma and parasitized duck erythrocytes in turkeys were conducted to test for the "sparing phenomenon" which Becker, Brodine, Marousek, and Byrd (4) discovered. The term sparing phenomenon was applied to the protective influence of the injected plasma in behalf of parasitized duck erythrocytes injected into chicks.

The blood plasma used in the experiments was prepared by the method previously described. The plasma was injected intravenously into test turkeys about an hour before the birds were injected with the parasitized erythrocytes, then again daily for 4 successive days after inoculation. Details of each experiment are covered under the discussion of each experiment.

Experiment 1

Both duck and turkey immune blood plasma were tested in this study. Tests were conducted in 3 groups of turkeys, duck plasma-recipient, turkey plasma-recipient, and untreated controls. Poults were 14 day old when injected.

Tables 17, 18, and 19 show the results of this experiment.

The results obtained in the duck plasma-recipients and in the controls (Tables 17 and 19) were compared by the Fisher (15) small sample method. A similar comparison was made of the turkey plasma-recipients and the controls (Tables 18 and 19). The variables were percentages of parasitized erythrocytes at certain times after inoculation. The format of this test for significance was provided by G. W. Snedecor (see Becker, Brodine, Marousek, and Byrd (4)). A solution of a problem by the Fisher small sample method is presented here for illustrative purposes. The percentages of parasitized cells in the duck plasma-recipients at 10 minutes after

Table 17

Percentage of Parasitized Cells in the Duck Plasma-Recipient Turkeys in Experiment 1 (Plasma Dose, 0.8 cc Per 100 g. Body Wt., Injected an Hour Before Inoculation, Then on Days 1, 2, 3, and 4; Parasite Dose, 2.5 x 10^8 P. C.; Ave. Wt. of Birds, 101 g.)

Time of Inoculation	Turkey No.							Average
	21	22	23	24	25	99	00	
10 Min.	0.73	0.50	0.83	0.76	1.00	0.40	0.73	0.70 ± 0.20
6 Hrs.	1.00	0.30	0.03	0.07	0.00	0.30	1.10	0.40 ± .46
1 Day	2.30	0.73	0.27	0.07	0.07	0.13	1.93	0.78 ± .94
2 Days	7.60	2.64	2.36	1.80	0.17	2.86	5.50	3.27 ± 2.47
3 Days	15.00	4.00	8.50	3.33	1.00	3.44	20.50	7.96 ± 7.22
4 Days	44.50	30.00	23.50	16.00	6.00	20.50	47.00	26.78 ±14.93
5 Days	64.00	23.50	45.50	36.50	3.10	28.50	65.00	38.30 ±22.08
6 Days	45.50	55.00	37.00	60.00	25.00	54.50	44.00	45.85 ±12.07
7 Days	35.00	35.00	15.00	34.50	48.00	50.00	19.50	33.85 ±13.07
8 Days	9.75	9.00	0.50	16.50	30.00	7.60	1.66	10.71 ±10.04
9 Days	5.60	3.31	0.03	0.63	45.50	0.60	0.20	7.98 ±16.67
10 Days	2.00	1.33	0.00	0.23	1.85	1.00	0.00	0.92 ± 0.85
11 Days	0.87	0.60	0.00	0.16	0.10	0.03	0.03	0.25 ± 0.34

Table 18

Percentage of Parasitized Cells in the Turkey Plasma-Recipient Turkeys in Experiment 1 (Plasma Dose, 0.8 cc per 100 g. Body Wt., Injected an Hour Before Inoculation, Then on Days 1, 2, 3, and 4; Parasite Dose, 2.5 x 10^8 P. C.; Ave. Wt. of Birds, 104 g.)

Time of Inoculation	Turkey No. 26	27	28	29	30	31	32	Average
10 Min.	0.00	0.07	0.17	0.53	0.57	0.57	0.57	0.35 ± 0.26
6 Hrs.	0.00	0.00	0.00	0.23	0.00	0.00	0.13	0.05 ± 0.09
1 Day	0.00	0.10	0.00	0.23	0.03	0.13	0.27	0.11 ± 0.10
2 Days	0.00	0.10	0.03	0.83	0.10	0.20	2.55	0.54 ± 0.92
3 Days	0.07	0.23	0.40	1.63	0.53	0.70	7.20	1.53 ± 2.54
4 Days	0.13	0.93	0.73	7.00	0.90	4.80	29.50	6.28 ±10.55
5 Days	0.23	1.85	2.17	6.60	1.70	3.25	29.50	6.47 ±10.34
6 Days	0.80	14.50	11.33	16.00	17.00	14.50	61.00	19.30 ±19.17
7 Days	6.20	19.50	25.00	18.50	31.00	24.50	54.00	25.52 ±14.72
8 Days	4.80	22.00	49.00	11.50	15.66	19.00	8.80	18.68 ±14.67
9 Days	7.40	43.50	36.00	0.03	29.50	1.57	0.10	16.87 ±18.81
10 Days	9.20	9.60	20.00	0.00	0.40	0.00	0.17	5.62 ± 7.69
11 Days	8.00	1.07	3.80	0.00	0.00	0.00	0.00	1.83 ± 3.05

Table 19

Percentage of Parasitized Cells in the Controls Receiving Injections of Physiological Salt Solution, in Experiment 1. (Dosages Same as With Turkey or Duck Plasma; Ave. Wt. of Birds, 104 g.)

Time of Inoculation	Turkey No. 90	94	95	96	97	98	Average
10 Min.	0.17	0.33	0.13	0.57	0.57	0.57	0.39 ± 0.20
6 Hrs.	0.07	0.03	0.00	0.20	0.07	0.30	0.11 ± 0.11
1 Day	0.03	0.07	0.00	0.33	0.17	0.20	0.13 ± 0.12
2 Days	0.20	0.33	0.03	0.73	0.40	0.53	0.37 ± 0.24
3 Days	0.27	0.80	0.60	5.80	1.27	2.50	1.87 ± 2.07
4 Days	3.13	12.50	2.27	18.25	2.78	7.00	7.65 ± 6.47
5 Days	2.32	10.00	6.44	12.75	5.20	15.50	8.70 ± 4.94
6 Days	13.00	29.00	20.50	46.00	20.00	34.50	27.16 ±11.90
7 Days	40.50	58.50	47.00	51.00	46.50	48.00	48.58 ± 5.94
8 Days	42.50	35.00	34.50	23.00	28.00	46.00	34.83 ± 8.60
9 Days	17.00	16.50	46.50	4.00	11.40	5.09	16.74 ±15.56
10 Days	7.80	2.04	18.00	4.80	2.12	1.13	5.98 ± 6.35
11 Days	0.07	0.73	10.70	2.14	1.10	1.70	2.47 ± 3.96

inoculation (Table 17) and in the controls (Table 19) constituted the variables of the groups to be compared. The complete procedure follows:

	Duck Plasma Treated Group	Control Group
Number of turkeys (n)	7	6
Degrees of freedom (n-1)	6	5
Sum of variables (SX)	4.95	2.34
Mean of variables ($\bar{x}$)	0.7071	0.3900
Sum of squares of variables (SX^2)	3.7423	1.1294
Sum x mean ($= (SX)^2/n$)	3.5004	0.9126
Sum of squares of dev. from mean (Sx^2)	0.2419	0.2168
Pooled (0.2419 + 0.2168)		0.4587
Divided by total degrees of freedom (6 + 5)		0.0417
Variance of mean difference ($s^2 = 0.0417 \times (1/7 + 1/6)$)		0.0129
Std. dev. of the mean difference ($\sqrt{s^2} = \sqrt{0.0129}$		0.1136
$t = (0.7071 - 0.3900) \div 0.1136$		2.7914*

In Fishers table of t, P (probability) for 11 degrees of freedom and t of 2.7914 gives a value of about 0.02, which indicates high significance. Is it to be concluded that the groups were different, and that the treatment with duck plasma produced an effect.

Results of the test for significance described previously are shown in Table 20 for the daily parasite counts between the duck plasma-recipients and the controls, and the turkey plasma-recipients and the controls. The table also summarises the records in the three groups of the mean percentages of parasitized cells on selected times after inoculation, up to 11 days. The data on mean percentage of parasitized cells summarized in this table presented in graphic form in Figure 10.

Experiment 2

A second set of experiment to test for "sparing action" of duck immune plasma in turkeys was made using 15-day-old turkeys of a different hatch. The rest of the procedure in the 1st experiment was followed.

The result of this series is shown in Tables 21 and 22 and are graphically presented in Figure 10. A test for significance between the duck plasma-recipients and the controls was made as in the previous experiment, and the results were tabulated in Table 23. The table also summarizes the mean per cent of parasitized cells at the time intervals after infection shown in the table.

General discussion of experiment 1 and 2. The differences in parasite counts between the means for the duck plasma-recipients and those for the controls in experiment 1 proved to be significant at 10 minutes, 6 hours, and 2 to

Table 20

Summary of the Mean Percentages of Parasitized Cells and Results of the Test of Significance Between the Duck Plasma-Recipients and Control, and Turkey Plasma-Recipients and Control, in Experiment 1 (P. C. = Parasitized Cells)

Time After Inoculation	Duck Plasma-Recipients			Control	Turkey Plasma-Recipients		
	P.	t	Mean % P. C.	Mean % P. C.	Mean % P. C.	t	P.
10 Min.	0.02	+2.79	0.70	0.39	0.35	-0.26	0.80
6 Hrs.	0.20	+1.48	0.40	0.11	0.05	-1.04	0.30
1 Day	0.10	+1.67	0.78	0.13	0.10	-0.38	0.70
2 Days	0.02	+2.84	3.27	0.37	0.54	+0.44	0.60
3 Days	0.05	+1.98	7.96	1.87	1.53	-0.25	0.80
4 Days	0.02	+2.90	26.78	7.65	6.28	-0.27	0.70
5 Days	0.01	+3.19	38.30	8.70	6.47	-0.48	0.60
6 Days	0.02	+2.79	45.85	27.16	19.30	-0.86	0.40
7 Days	0.05	-2.53	33.85	48.58	25.52	-3.57	0.01
8 Days	0.01	-4.60	10.71	34.83	18.68	-2.36	0.05
9 Days	0.30	-0.97	7.98	16.74	16.87	+0.01	0.90
10 Days	0.10	-2.09	0.91	5.98	5.62	-0.09	0.90
11 Days	0.10	-1.66	0.25	2.74	1.83	-0.46	0.60

Figure 10. Graphic presentation of mean parasite counts in experiments 1 and 2, comparing duck plasma-recipients and turkey plasma-recipients with their controls.

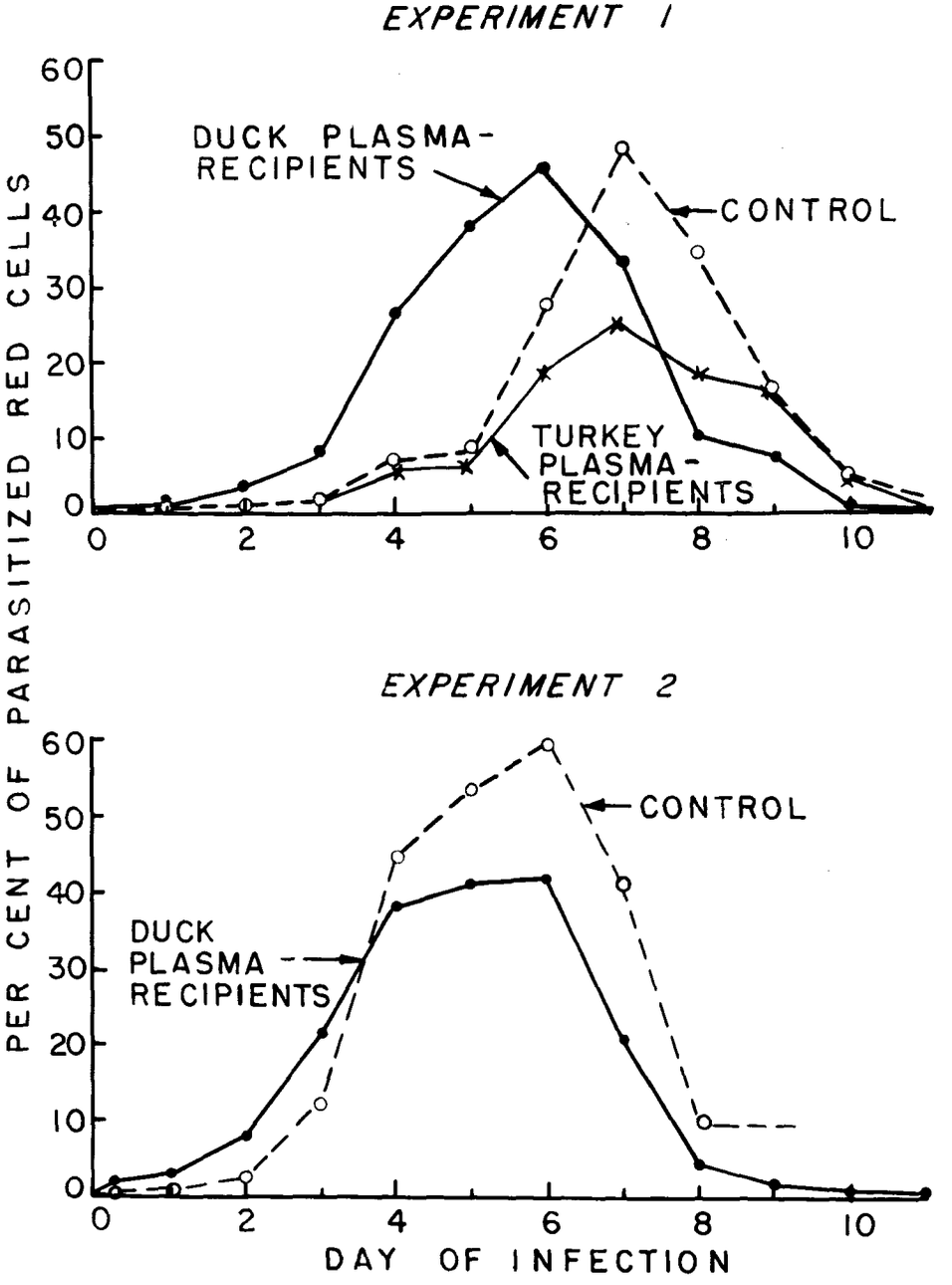

EXPERIMENT 1
DUCK PLASMA-RECIPIENTS
CONTROL
TURKEY PLASMA-RECIPIENTS
EXPERIMENT 2
CONTROL
DUCK PLASMA-RECIPIENTS
PER CENT OF PARASITIZED RED CELLS
DAY OF INFECTION
0
10
20
30
40
50
60
0
2
4
6
8
10

Table 21

Percentage of Parasitized Cells in the Duck Plasma-Recipient Turkeys in Experiment 2. (Plasma Dose, 0.8 cc Per 100 g. Body Wt., Injected an Hour Before Inoculation, Then on Days 1, 2, 3, and 4; Parasite Dose, 2.5 x 10^8 P. C.; Ave. Wt. of Birds, 108 g.)

Time After Infection	Turkey No. 76	77	78	79	80	81	82	83	Mean
10 Min.	0.70	1.03	0.77	0.60	0.63	0.63	0.50	0.53	0.67 ± 0.16
6 Hrs.	1.70	3.84	3.44	2.00	1.30	0.50	2.00	2.10	2.11 ± 1.08
1 Day	2.90	7.20	4.20	6.80	2.03	0.60	2.77	4.60	3.88 ± 2.28
2 Days	5.00	8.80	7.00	10.00	5.40	2.20	10.00	20.50	8.61 ± 5.50
3 Days	8.80	26.50	29.00	30.00	8.33	3.33	26.50	38.50	21.37 ±12.71
4 Days	30.70	61.00	51.00	48.50	23.00	1.46	38.50	51.00	38.77 ±19.78
5 Days	28.00	69.00	60.00	52.00	12.33	3.00	40.00	66.00	41.29 ±24.86
6 Days	56.00	54.00	67.00	37.00	37.00	16.00	20.33	52.00	42.44 ±17.96
7 Days	32.00	Death	27.00	5.66	36.00	24.00	4.12	25.00	21.96 ±12.38
8 Days	2.00		1.80	1.40	7.14	14.66	0.60	7.85	5.06 ± 7.89
9 Days	0.33		0.10	0.10	0.30	8.66	0.10	4.00	1.94 ± 3.28
10 Days	0		0	0	0	0.10	0	0.10	0.02 ± 0.04
11 Days	0		0.07	0	Death	0	0.07	1.66	0.30 ± 0.66

Table 22

Percentages of Parasitized Cells in the Controls Receiving Injections of Physiological Salt Solution, in Experiment 2. Dosages Same as With Duck Plasma (Average Weight of Birds, 147 g.)

Time After Infection	Turkey No. 43	44	45	46	47	48	49	50	Mean
10 Min.	0.43	0.33	0.37	0.47	0.33	0.43	0.47	0.50	0.41 ± 0.06
6 Hours	0.27	0.20	0.30	0.50	0.43	0.66	0.43	0.70	0.43 ± 0.17
1 Day	0.40	0.80	1.36	1.73	1.16	1.20	1.50	0.80	1.11 ± 0.43
2 Days	2.00	1.90	4.80	4.00	1.60	4.20	7.00	4.00	3.68 ± 1.81
3 Days	2.17	12.66	26.50	15.50	13.00	12.50	12.33	13.33	13.49 ± 6.59
4 Days	21.00	36.50	69.00	64.50	27.50	53.00	49.50	40.00	45.12 ±16.98
5 Days	28.00	42.00	74.00	70.00	56.00	60.00	44.00	65.00	54.87 ±15.72
6 Days	65.00	Death	75.00	Death	59.00	58.00	44.00	Death	60.20 ±11.30
7 Days	61.00		Death		30.00	34.00	40.00		41.25 ±13.79
8 Days	Death				12.66	8.00	11.66		10.77 ± 2.45
9 Days					12.66	7.40	11.00		10.35 ± 2.68
10 Days					8.40	25.00	19.50		17.63 ± 8.45
11 Days					20.00	44.00	70.00		44.66 ±25.00

Table 23

Summary of Mean Percentages of Parasitized Cells and Results of Test for Significance Between the Duck Plasma-Recipients and Control, in Experiment 2 (P. C. = Parasitized Cells)

Time After Infection	Duck Plasma-Recipients % P. C.	Control % P. C.	t	P.
10 Min.	0.67	0.41	+4.04	0.01
6 Hrs.	2.11	0.43	+4.31	0.01
1 Day	3.88	1.11	+3.36	0.01
2 Days	8.61	3.68	+2.40	0.02
3 Days	21.37	13.49	+1.55	0.10
4 Days	38.77	45.12	-0.68	0.50
5 Days	41.29	54.87	-1.30	0.20
6 Days	42.41	60.20	-1.96	0.05
7 Days	21.96	41.25	-2.38	0.02
8 Days	5.06	10.77	-1.79	0.05
9 Days	1.94	10.35	-3.87	0.01
10 Days	0.02	17.63	-6.03	0.01
11 Days	0.30	44.66	-4.68	0.01

8 days (Table 20, Figure 10). The mean of 3.27 per cent parasitized cells for the duck plasma-recipients on the 2nd day is almost 9 times as high as that of 0.37 per cent parasitized cells for the controls. While the means of the former group on the 1st day is about 7 times as high as that of the latter, the difference only came close to being significant since P 0.10 was obtained. The peaks of parasitemia in both groups reached about the same heights, and the decline was at about the same rate.

The turkey plasma-recipients were not significantly different from the control up to the 6th day. The peak of parasitemia was reached in both groups on the 7th day. The differences between the means of parasite counts in the two groups on this day as well as on the 8th day proved significant. The parasite percentage in the turkey plasma-recipients was held at a low level throughout the course of infection.

In experiment 2 (Table 23, Figure 10), the difference between the means of parasite percentage in the duck plasma-recipients and that of the controls at 10 minutes, 6 hours, 1 day, and 2 days proved significant. In the controls there was a rise in parasite counts up to the peak on the 6th day. The turkey plasma-recipients also reached their peak on the 6th day, but the parasite counts were held at a much lower level compared to the controls from the 4th day.

From the data obtained in the foregoing experiments

the following is indicated: (1) the immune duck plasma exerted an initial sparing action in behalf of the parasitemia in turkeys induced with parasitized duck erythrocytes, and it conferred on the host partial protection against the primary attack after this sparing action has ceased; (2) turkey immune plasma conferred partial protection, that is, a degree of passively acquired immunity, on the host throughout the general course of infection; and (3) immune duck plasma conferred a passively acquired protective effect in turkeys inoculated with parasitized duck cells.

The immunity experiments in turkeys present further proof of the sparing phenomenon and protective action of immune duck plasma in behalf of the parasitemia induced with parasitized duck erythrocytes. Becker, Brodine, Marousek, and Byrd in 1949 (4) proved that in chicks immune and normal duck plasma may exert a sparing action on the removal of duck erythrocytes, parasitized with Plasmodium lophurae, from the peripheral circulation of chicks. The authors found the potency of this action to be in some cases practically nil, in others it was more or less significantly strong, while in certain cases it was exceedingly strong. Experiment 1 of the present study seemed to fall into the 3rd category, while experiment 2 was of the 2nd.

As shown in Figure 10, the infection in all groups was characterized by a moderately acute rise in parasitemia to

the peak of the infection, which was terminated by a moderately sharp crisis. This crisis was usually followed within a few days by latency. Taliaferro and Taliaferro (37) reported that latency in chickens is ordinarily not interrupted by relapses of parasitemia. In experiment 2 of this study, however, the control groups had relapses of parasitemia.

It seems apparent that the sparing action exhibited by duck plasma against parasitized duck erythrocytes during the initial stages of infection soon becomes interrupted by the protective properties of the duck plasma, as manifested by the acute decline in parasitemia.

In this study, immune duck plasma showed an initial sparing action, although in experiment 2, as shown in Figure 10, the protective effect became evident early, on the third day of the infection.

Becker et al (4) further stated that this sparing action of duck plasma on the removal of duck cells, parasitized with Plasmodium lophurae, from the circulating blood of the chick may be of significance, not only as a partial explanation of relapse in lophurae-malaria, but also of the physiological problem of why machrophages of certain organ of the body, as the spleen, do not normally phagocytose normal erythrocytes while removing misshapen, effete or diseased erythrocytes from the circulation.

A later study on the specificity of the sparing phenomenon in 1950 by Becker, Schwink, Byrd, and Conn (8) has

brought out the fact that the sparing phenomenon cannot be interpreted as (1) a non-specific protein effect, (2) an illusion due to hemolysis, (3) blocking of the macrophages in the ordinary sense, (4) a state of intoxication of the host produced by heterologous plasma, (5) an effect produced by the anticoagulant, or (8) a reduction of the lag phase in reproduction of a parasite not yet adapted to its new specific host, brought about by an unknown but essential (to the parasite) quality of duck plasma. Since the facts do not bear a possible alternative explanation, the authors concluded that the effect must be regarded as specific, although not species specific.

As may be noted in the procedure, in the immunity experiments, the plasma was injected an hour before inoculation, then on the 1st, 2nd, 3rd, and 4th day afterwards. Becker et al (6) proved in their experiment that birds receiving a single dose of 0.8 cc of guinea plasma were more susceptible to infection than those that received 3 additional injections. These observations are not in comfermity with the well-known cumulative effects of repeated injection, and in one way rule out the possibility of sparing effect being due to blocking.

That immunity can be passively transferred to normal chickens, provided sufficient doses of immune serum (from latently infected chickens) are used and continued over a

sufficient period, was reported by Taliaferro and Taliaferro in 1940 (37) for Plasmodium lophurae. In successful experiments serum was given for 9 days, which included the period of the acute rise on the infection. Becker et al in 1949 (5) also proved this fact of immune duck plasma on chicks inoculated with parasitized duck cells in Plasmodium lophurae infection.

If the sparing effect were attributed to a general breakdown of the host's resistance produced by the toxicity of duck plasma, other manifestations such as inappetence and consequent failure to grow or to gain in weight should appear. In order to control this possibility, all turkeys in both experiments were weighed before the infection, then daily or every other day up to the end of infection.

Table 24 gives a summary of the average daily weights of the various groups in both series of experiments.

It may be noted that from the daily gain in weight recorded in Table 24 there is no evidence which suggests that the plasma-recipients in general made less growth gains than their controls which received saline solution. In experiment 1, growth made during the 1st week of infection by the plasma-recipients was less than that by the controls. This was undoubtedly due to the presence of much higher parasitemia in treated groups. However, the second week's gains showed the reverse, the plasma-recipients having gained

Table 24

Mean Daily Weight in Grams of Turkeys in Each of the Groups of Experiment 1 and 2 of the Immunity Test Series

Item	Duck Plasma-Recipients	Control	Turkey Plasma-Recipients
Experiment 1:			
No. Turkeys	7	6	7
Day After Infection:			
0	101.57	104.16	104.28
1	106.71	115.83	115.85
2	116.14	129.00	126.71
3	124.58	136.50	133.28
4	128.28	151.83	144.43
5	138.28	151.83	153.86
6	140.28	171.32	163.86
7	142.00	181.17	157.43
14	220.83	232.00	246.33
Average Daily Gain From 1 - 7 Days	5.89	11.09	7.75
Average Daily Gain From 7 - 14 Days	11.26	7.26	12.70
Average Daily Gain From 1 - 14 Days	8.57	10.24	9.17
Experiment 2:			
No. Turkeys	8	8	
Day After Infection			
0	108.75	147.25	
2	131.88	163.13	
4	143.88	164.38	
6	147.75	140.13	
8	161.86	147.50	
10	182.86	162.00	
13	200.83	194.00	
15	198.62	207.00	
17	228.10	224.50	
Average Daily Gain From 1 - 8 Days	13.28	5.06	
Average Daily Gain From 8 - 17 Days	15.08	20.83	
Average Daily Gain From 1 - 17 Days	14.18	12.94	

in weight at a higher rate. Over a two-week period, hardly any difference can be detected between the groups. Mortality in all three groups was about the same.

In experiment 2, the plasma-recipients made better growth gains during the 1st week and also over a 17-day period. Mortality was higher in the control group. The reverse result could have been expected had the plasma been toxic.

In the recent studies of Becker, Schwink, and Brodine (8) on sparing and protective factors in plasma fractions of ducks recovered from lophurae-malaria, it was shown that the bulk of the sparing factor in immune duck plasma is concentrated in the protein fraction precipitate by amonium sulphate in the 33.4 - 50% saturation range, while the bulk of the factor that conferred a degree of passive immunity is concentrated in the protein fractions precipitated by ammonium sulphate in the 0% to 33.3% saturation range, and that by selecting samples it was possible to obtain fractions of plasma potent in protective factor and weak or lacking in sparing factor, and vice versa.

The in vitro counterpart of the sparing phenomenon, viz., inhibition of hemagglutinins for parasitized duck erythrocytes in the plasma of chicks recovered from lophurae-malaria, was reproduced by Becker, Swink and Prather (9) in 1951. They reported also that the sparing phenomenon is not the result of creation, by a super-abundance of hemagglutinin,

of such large concentration of erythrocytes that they cannot be phagocytesed.

Exoerythrocytic Stages

According to Taliaferro and Taliaferro (39), Porter and Laird in a personal communication reported they had not found exoerythrocytic stages of Plasmodium lophurae in blood-induced infection of Plasmodium lophurae. In a list of species of Plasmodium for which "phanerozoites" were known, Huff (26) did not list Plasmodium lophurae. Huff and Coulston (30) proposed the term "phanerozoite" for exoerythrocytic stages of malaria parasites which occur late in the infection and not as one of the pre-erythrocytic stages.

Phanerozoites were first found in brain smears of two 46-day-old turkeys which succumbed on the 21st day of infection with Plasmodium lophurae. The infections were induced with 5×10^7 parasitized turkey erythrocytes per 100 g. body weight.

Smears of the brains of the diseased birds were fixed in methyl alcohol and stained in Giemsa. Phanerozoites measuring from $7.1\mu \times 19.4\mu$ to $13\mu \times 69.3\mu$ were observed in the capillaries. The capillaries were obviously occluded by the larger parasites, and in some cases were definitely distended by restrained erythrocytes. The relationship of

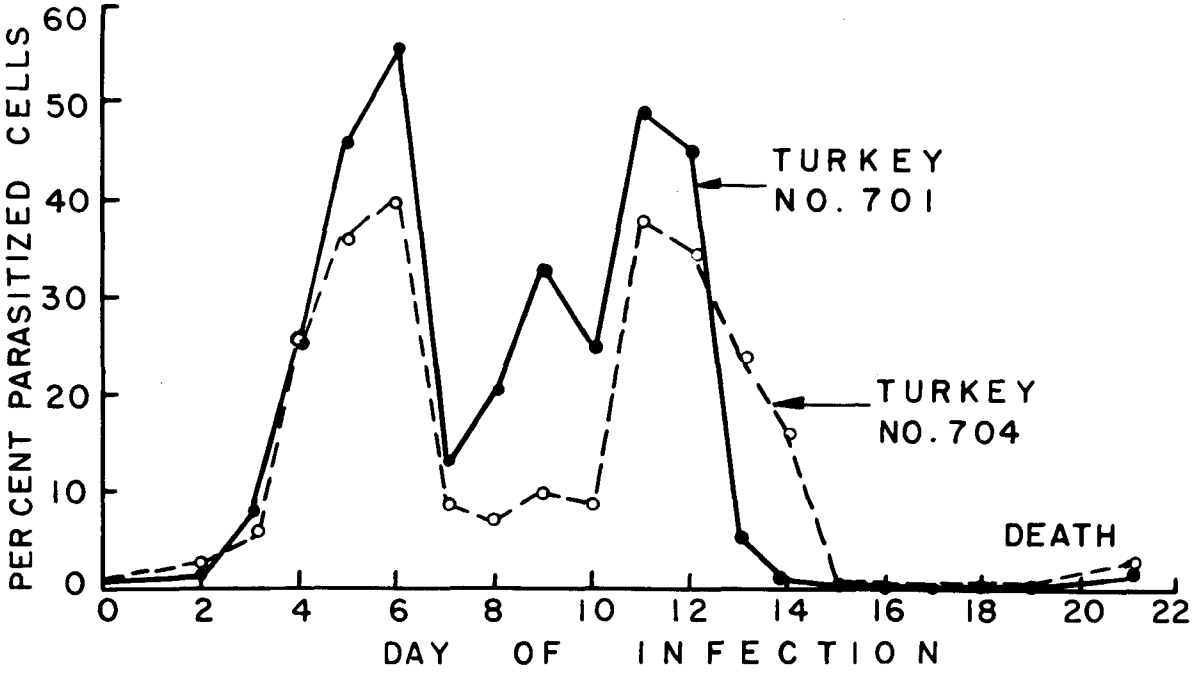
the parasites to the capillary was very much like that observed by James and Tate in 1938 (32) in Plasmodium gallinaceum infection of chickens. They reported that this blockage of the capillaries resulted in symptoms of general paralysis in the infected birds and death within a few days.

Figure 11 shows the course of infection in the two turkeys in which phanerozoites were found. It may be noted that the parasitemias attained 56% and 40%, respectively, on the 6th day, then subsided, attained new peaks at 50% and 39%, respectively, on the 11th day, and subsided to 0% on the 17th and 18th days. A few parasites were seen in the blood again on the 19th day. The turkeys were found dead on the morning of the 21st day. Post-mortem blood smears showed a parasitized cell incidence of 1.83% and 2.70%, respectively.

In view of the foregoing discovery of exoerythrocytic stages of Plasmodium lophurae in the brains of turkeys, further studies were conducted to learn the general characteristics, behavior, and occurrence of the parasitic stages in the brains of turkeys. Smears prepared of the spleen, liver, kidney, heart, and lung in all turkeys that died, as well as those that were killed for routine examination, failed to show the presence of the stages. Only in the brain smears were they found.

Studies of James and Tate in 1938 (32) showed that when exo-erythrocytic stages were found in birds during actue in-

Figure 11. Course of infection with Plasmodium lophurae in turkeys where phanerozoites were found.


PERCENT PARASITIZED CELLS
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
DAY OF INFECTION
TURKEY NO. 701
TURKEY NO. 704
DEATH

fections or relapse, the degree to which various organs are involved was very variable. The brain and spleen were the organs most commonly infected, next came the liver, kidney, heart, lung, and, lastly, the bone marrow.

Incidence of exoerythrocytic stages

To determine the time at which phanerozoites were most abundant in the brain and to find the range of the time over which they appeared after infection, brain smears were made of all birds that died of the infection and also of birds killed at various time intervals after infection. A total of 88 turkeys were examined for exoerythrocytic stages. The results are presented in Table 25.

For lack of a better measurement of the intensity of exoerythrocytic infection, the present method of measurement, called the EE intensity index was devised. One hundred lengths of blood vessels traversing the field of the oil immersion (97x) lens were counted successively, and the total number of schizonts in these 100 lengths recorded to represent the intensity of exoerythrocytic infection. Haas, Wilcox, Laird, Ewing, and Coleman in 1948 (18) employed mortality of chicks as the criterion of intensity of exoerythrocytic population in gallinaceum malaria. Since it is believed that some of the turkeys with exoerythrocytic forms in the brain would have recovered, the method used here undoubtedly gave more accurate results.

Table 25

Incidence of Exoerythrocytic Stages in Brain Smears of 88, 6 to 32-Day-Old Turkeys. EE Intensity Figure in the Last Column of the Table Represents the Number of Schizonts in 100 Microscopic Field Lengths of Blood Vessels (EE = Exoerythrocytic)

Day After Infection	No. of Turkeys Examined	Result of EE Examination		
		No. of Negative	No. of Positive	EE Intensity Index
6	7	7		
7	6	6		
8	2	2		
9	2	2		
10	2	2		
11	2	2		
12	3	3		
13	2	2		
14	2	2		
15	4	2	2	76
16	2	2	0	0
17	5	2	3	128
18	5	3	2	235
19	7	1	6	358
20	7	0	7	239
21	5	1	4	214
22	4	3	1	118
24	4	3	1	102
25	2	2		
26	2	2		
27	2	2		
28	2	2		
29	2	2		
30	2	2		
31	2	2		
41	2	2		
97	1	1		

As shown in Table 25, the phanerozoites found in brain smears occurred around the 15th to the 24th days after infection, at which time the erythrocytic infection had become negative, or almost so. The greatest incidence occurred on the 19th, 20th, and 21st day after infection, as shown by the intensity indices of 358, 239, and 214, respectively, and the number of turkeys that were positive for exoerythrocytic forms.

Results of similar observation in Plasmodium gallinaceum by Haas, Wilcox, Davis, and Ewing in 1946 (17) showed cases in which the exoerythrocytic forms predominated, and that death due to them occurred about 11 days after inoculation.

James and Tate (32), in reporting the exoerythrocytic forms of Plasmodium gallinaceum in 1938, stated that there was great irregularity in the period of the infection during which exoerythrocytic stages developed. The results of the present study on exoerythrocytic stages showed that the cycle occurred from about the 15th to the 24th day, or after erythrocytic stages had cleared. This observation is in agreement with the results reported by Haas, et al, in 1948 (18) from their study of the different responses of exoerythrocytic forms to alteration in the life-cycle of Plasmodium gallinaceum. They found 3 general patterns, the 2nd of which occurred in chicks infected by blood-inoculation. They reported that there was an acute stage, marked by heavy erythrocytic infection. Many chicks died during this period,

but exoerythrocytic forms became prevalent about the 3rd week after inoculation in those that survived, often with fatal results.

Effect of sex, plasma, and parasitemia on exoerythrocytic forms

The data for this study were taken from the same set of turkeys used for the immunity studies under experiment 1. This consisted of examining stained brain smears for exoerythrocytic forms of all turkeys in the duck plasma-recipients, turkey plasma-recipients, and the controls, at intervals of a number of days after inoculation, or at the time of death. The birds were sexed at post mortem. Exoerythrocytic forms when present were rated in intensity by the method previously described. Results of the foregoing study appear in Table 26.

As shown in Table 26, of the 19 turkeys studied (8 males and 11 females), 6 males and 9 females showed phanerozoites in brain smears. These data seem to indicate that sex did not have any perceptible effect on exoerythrocytic formation.

That the effect of duck or turkey plasma did not influence exoerythrocytic formation is shown by the fact that 5 turkeys in each of the duck plasma-recipients, turkey plasma-recipients, and controls gave positive results for phanerozoites. The "EE index" given for the positive counts represents the number of schizonts in 100 microscopic field

Table 26

Effect of Sex, Plasma, and Parasitemia on Exoerythrocytic forms. EE Intensity Index Expressed as Number of Schizonts in 100 Microscopic Field Lengths of Blood Vessels. (EE = Exoerythrocytic; PM = Post Mortem)

Day After Infection	Turkey No.	Sex	% Parasitized Cell At Peak	% Parasitized Cell On Day of EE Exam.	Result of EE Examination: Duck Plasma-Recipients Negative	Duck Plasma-Recipients Positive (EE Index)	Control Negative	Control Positive (EE Index)	Turkey Plasma-Recipients Negative	Turkey Plasma-Recipients Positive (EE Index)
12	22	M	55.00	0.10	--					
12	32	M	61.00	0.27			--			
12	94	F	58.00	0.20					--	
17	00	M	65.00	3.60		290				
17	28	F	49.00	0.03						35
17	96	F	51.00	0.30				52		
18	98	F	48.00	1.00				240PM		
19	21	F	64.00	3.33		310				
19	30	F	31.00	0.10						210
19	95	M	46.00	0.17				150		
19	97	F	46.00	0.17				222		
20	23	M	45.00	5.40		284PM				
20	29	M	18.50	4.60						325PM
20	31	F	24.50	1.70						263PM
20	24	M	60.00	1.33		161				
20	27	F	43.50	0.00						276
21	90	F	42.50	0.10				228		
22	25	M	48.00	3.60		118				
30	99	F	31.80	0.53			--			

lengths of blood vessels, the method of determination of which has been described previously. The EE indices for the 15 positive turkeys varied in intensity since they were taken at various intervals after inoculation. These EE indices were presented here merely to show the relative strength or intensity of exoerythrocytic infection in the groups at various days after inoculation.

It will also be noted from Table 26 that all the turkeys tested for exoerythrocytic forms had been cleared of parasitemia stages or nearly so at the time they died of the exoerythrocytic infection or were killed for brain smears. This result is also in full accord with that reported by Huff and Coulston in 1946 (30) in their work with Plasmodium gallinaceum in chickens wherein they found that when malaria was produced by the inoculation of parasitized erythrocytes, exoerythrocytic stages (phanerozoires) appeared late in the infection, usually reaching a maximum in numbers during the 3rd or 4th week. The phanerozoite stages gradually increased in number beginning at about the time of the crisis of the parasitemia. Huff and Coulston further pointed out that this difference in point of time of the maximum number of parasitized erythrocytes and the number of phanerozoites was of great theoretical importance, for it demonstrated an almost complete independence of the two components of the blood-induced avian malarial life cycle. That these two

cycles are related was demonstrated by Coulston and Manwell in 1941 (13) for Plasmodium circumflexum and by Downes in 1947 (14) for Plasmodium gallinaceum when they injected single parasitized erythrocytes into birds which developed both parasitemia and phanerozoites.

SUMMARY AND CONCLUSIONS

The present studies were undertaken to determine the status of the turkeys as a host for Plasmodium lophurae. Among the subjects studied were (1) age of turkeys and dosage of turkeys and dosage of infection, (2) biological characteristics and host-parasite relationships, (3) characteristics of the infection, (4) merozoite production, survival and death rates of the parasite during the course of primary infection and relapse, (5) immunity, and (6) exo-erythrocytic stages.

Age of Turkeys and Dosage of Infection

1. Age resistance apparently does not develop in turkey poults up to 32 days of age.

2. Dosage of from 0.5 to 2.6 x 10^8 parasitized cells per 100 g. of the body weight of the bird seemed satisfactory for the study of the parasite in turkeys.

Biological Characteristics and Host-Parasite Relationships

Biological characteristics

1. The morphological characteristics of the organism are in accordance with the descriptions given by Coggeshall in 1938 (10) in his paper describing Plasmodium lophurae in chicks, and by Hewitt (22) in ducks.

2. Phanerozoites were found in smears of brain tissue,

and studies on these stages reported.

3. Examination of the smears of the spleen, liver, kidney, heart, and lung of all turkeys that died or were killed failed to show the presence of exoerythrocytic stages.

4. Periodicity studies were conducted over an interval of 3 to 5 days on blood smears made every 3 to 6 hours. The method of study is described, and on the basis of the data obtained the following conclusions were drawn:

(a) The degree of synchronicity of Plasmodium lophurae in the turkey is low.

(b) The length of the asexual cycle in the turkey is established at 36 hours.

(c) Further analysis of the data on periodicity show that majority of the merozoites succeed in penetrating new red cells, without discriminating against immature erythrocytes.

5. Experiments on latency indicate that although the blood of turkeys recovered from erythrocytic infection remained negative for days after the initial infection, the parasite could be demonstrated for 97 days (longest period tested) by sub-inoculation of large quantities of blood into normal turkeys.

Effect of the host on the length of the asexual cycle

1. The length of asexual cycle in the duck-duck infections is established at 36 hours, 39 hours in the duck-turkey infections, and reverts to 36 hours in the turkey-turkey infections.

2. In turkeys with fatal infections, shortening of the length of the last asexual cycle is indicated, in these cases from the usual 36 hours to 27 hours (prior to death).

Temperature Studies

1. Rectal temperature of unparasitized turkey poults ranged from 105.0°F to 107.8°F, with a mean of about 106.45°F ±0.24.

2. In general there is little difference in rectal temperatures between the parasitized and unparasitized turkeys for the 1st to the 4th or 5th days after inoculation.

3. A sudden drop to sub-normal temperature occurs just about a day prior to the peak in parasitemia on the 6th day, remains sub-normal for a day or two, and again goes back to within normal range as the parasitemia declines.

4. Sub-normal temperature precedes death.

5. Further analyses of the data obtained in temperature studies and those of periodicity studies show none of the temperature can be correlated with an increase in any particular growth stages.

Merozoite Production, Survival and Death Rates of *Plasmodium lophurae* During Primary Infection and Relapse

1. Initial infection and relapse of *Plasmodium lophurae* have the same length of asexual cycle, 36 hours, in turkeys. They, however, differ markedly with respect to the rate of reproduction and survival and death of parasites.

2. In turkeys the over-all survival rate of the parasite is low for the 1st segmentation of the initial infection being only 26.10%, 26.43%, and 25.16% for the 3 turkeys studied. There is a gradual decline in survival rate in succeeding segmentations until the average number of merozoites that survived per asexual cycle was less than 1. Similar observations are noted in relapses, and, furthermore, the parasite survival is proportionally reflected in the intensity of relapse.

3. Evidence of acquired active immunity appears progressively against *Plasmodium lophurae* in turkeys.

4. Results of the study give further direct proof that much of the reduction in number of merozoites produced per segmenter, which reaches its maximum near the crisis, is due to acquired immunity.

Immunity Experiments

1. Immune duck plasma exerted an initial sparing action in behalf of the parasitemia in turkeys induced with parasitized duck cells, and it conferred on the host partial protection against primary attack soon after the sparing effects ceased to be apparent.

2. Immune turkey plasma conferred partial protection on the host throughout the general course of infection.

3. Immune duck plasma may confer a passively acquired immunity on turkeys inoculated with parasitized duck cells.

4. From the daily growth gains of the birds, there is no evidence which suggest that the plasma-recipients made less growth gains than their controls. Thus, the possibility of sparing action being due to a result of a state of intoxication produced in the host is ruled out.

5. The present study in turkeys is in full accord with the previously reported sparing action effect of duck plasma on duck cells injected into chicks.

Exoerythrocytic Stages

In view of the lack of better means to measure the intensity of exoerythrocytic infection, a method was devised for use in the present study. This method gives a measure of the intensity of exoerythrocytic infection in terms of the number of schizonts in 100 microscopic field lengths of

blood vessels, and is being expressed as "EE Intensity Index".

From the data obtained in this study it is possible to report the following preliminary observations.

1. Phanerozoites of Plasmodium lophurae occur in the brain of turkeys around the 15th to the 24th days after infection. The greatest intensity of exoerythrocytic stages in the brain occur around the 19th day after infection (EE Intensity Index on this day is 358).

2. In turkeys, the exoerythrocytic stages occur in birds that survive the erythrocytic infection.

3. Age, immune plasma, and intensity of parasitemia apparently do not affect the development of exoerythrocytic stages.

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APPENDICES

Appendix 1

Chick Starting Mash

Guaranteed Analysis

Crude Protein, not less than	20.00%
Crude Fat, not less than	3.00%
Crude Fiber, not more than	7.50%
Minerals	4.25%

Ingredients

Ground yellow corn, wheat middlings, wheat bran, ground oats, soybean oilmeal, meat scrap, dehydrated alfalfa meal, dried buttermilk, vitamin A and D feeding oil, D-activated animal sterol (source of vitamin D3), limestone, salt.

Also: 2.50% Dr. Hess Feed Builder for Poultry which contains:

Bone black, steamed bone meal, defluorinated phosphate, animal protein factor supplement (source of vitamin B12 activity), rock phosphate, iron sulphate (copperas), iron oxide, manganese sulphate, dried grains and skimmed milk fermentation solubles (source of riboflavin), copper sulphate, potassium iodide.

Appendix 2

Rules for Counting Malarial Parasites
(After Ginrich, Jour. Prev. Med. 6:197-246. 1932.)

1. If 0, 1, 2, 3, 4, or 5 parasitized cells are found in the first 100 red cells counted, proceed either until 3000 red blood cells or 50 parasitized cells have been counted. (If no parasitized cells are encountered in the first 3000 red blood cells, continue until a parasitized cell is found, or until 10,000 red cells have been counted).

2. If 6 thru 10 parasitized cells were present, count 500 red blood cells.

3. If 11 thru 13 parasitized cells were present, count 400 red blood cells.

4. If 14 thru 17 parasitized cells were present, count 300 red blood cells.

5. If 18 or more parasitized cells were present, count 200 red blood cells.

The per cent of infected cells is computed directly by dividing the number of parasitized cells by the number of hundred of red blood cells counted.

Example 1.

> 36 parasitized cells were present in 3,000 red blood cells counted.
>
> Per cent parasitized cells is 36 over 30 or 1.2%.

Appendix 2 (Continued)

Example 2.

50 parasitized cells were present in 2,970 red blood cells counted.

Per cent parasitized cells is 50 over 29.70 or 1.7%.

Example 3.

48 parasitized cells were present in 400 red blood cells counted.

Per cent parasitized cells is 48 over 4 or 12.0%.

Select a field about 1/4 inch from the extremity of the tongue end of the blood smear where the red blood cells are evenly distributed and number about 100. The number of parasitized cells in the first 100 red cells can be used to give an approximation of the number of red cells that must be counted to give a parasite estimation with a probable error of 10%. This does not allow for non-random factors in the distribution of parasitized cells and does not hold for counts below 150 parasitized cells per 10,000 red blood cells.