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Mechanism of Spring Relapse in Avian Malaria: Effect of Gonadotropin and Corticosterone

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Abstract

Previous work demonstrated that corticosterone induces relapse of avian malaria, and that this effect varies markedly from winter to spring. In the present study, English sparrows with latent *Plasmodium relictum* infections were treated in winter with corticosterone, gonadotropin, corticosterone + gonadotropin, or a control regimen consisting of the oil vehicle. Gonadotropin neither induced relapse nor potentiated the induction of relapse by corticosterone. These data cast doubt on the hypothesis that spring relapse in malarial infections is mediated by seasonal changes in reproductive hormone levels.

Introduction

Sparrows infected with *Plasmodium relictum* experience a relapse in the spring.[2,4] This phenomenon appears to occur synchronously throughout the population of infected birds coincident with the onset of spring reproductive activity. In previous work,[1] we found that corticosterone injections elevated parasite populations in established infections. We also noted that the response to corticoid treatment increased as season advanced from winter to spring, and that the level of spontaneous relapse in control birds likewise increased from January to April. Our data suggested that adrenal cortical activity might be involved in spring relapse, but that some other factor, possibly connected with the reproductive cycle, was contributing also. The purpose of the present study was to test the effect of artificial gonadal stimulation and corticosterone treatment on sparrow populations having established malaria infections.

Materials and Methods

The study occurred during December, 1969 and January, 1970, at the Naval Medical Research Institute, Bethesda, Maryland. The experimental host was the English sparrow *(Passer domesticus)*. Sparrows were captured in late November at State College, Pennsylvania, from a population in which the prevalence of natural *Plasmodium relictum* infections was estimated to be 73.3% by isodiagnosis. Infections in the birds used in this study were ascertained by the same technique.

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The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, NAS-NRC.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

In the laboratory, sparrows were kept in 12x10x16-inch cages and fed finch seed daily. Light was controlled at a constant 12-hour cycle from 6:00 a.m. to 6:00 p.m. A temperature of 24 C was maintained in the laboratory.

Corticosterone used in this study was obtained from Calbiochem. A dosage of 0.25 mg/bird/day (= 10 mg/Kg) was prepared and administered according to methods previously described[1]. The gonadotropic substance used was Equinex(R), a serum gonadotropin from Ayerst Laboratories. A dosage of 25 IU/bird/day dissolved in 0.05 ml sterile saline diluent was administered intramuscularly in the breast. Control birds received 0.05 ml peanut oil.

Blood was drawn by clipping a toenail. Thin blood films were fixed in methyl alcohol, stained in Giemsa's stain and examined under oil immersion (970X). Each blood film was examined 5 minutes, during which time approximately 40,000 RBC's were scanned.

The experimental design involved four groups of five sparrows each, treated as follows: (I) corticosterone, (II) gonadotropin, (III) corticosterone + gonadotropin, and (IV) oil control. Birds receiving gonadotropin were given this compound daily for 14 days prior to the onset of the experiment. During the experiment birds were treated with gonadotropin every other day from day 1 through day 10. Corticosterone and oil were administered daily from day 1 through day 10. All birds were bled at 48-hour intervals from day 1 through day 20.

The chi-square method was used for statistical analysis of data.

Results

The stimulatory effect of serum gonadotropin on house sparrow gonads has been demonstrated.[9] In the present study a 400-500-fold difference in the weight of sparrow testes was noted between treated and nontreated birds. Using gonadal weight as a criterion, the regimen of gonadotropin used in this study simulated reproductive changes normally found in spring.

TABLE 1. *Effect of corticosterone and gonadotropin on parasite populations in established infections.*

Group	Treatment	No. Birds	% Birds Relapsing	No. Blood Films	% Blood Films w/parasites
I	Corticosterone	5	60	40	30.0**
II	Gonadotropin	5	40	49	10.2
III	Corticosterone + Gonadotropin	5	80	50	22.0*
IV	Oil Control	5	20	50	6.0
II, III	Gonadotropin Gonadotropin and + Corticosterone	10	60	99	16.2
I, IV	Corticosterone, and Oil Control	10	40	90	16.7
I, III	Corticosterone, and Corticosterone + Gonadotropin	10	70	90	25.6
II, IV	Gonadotropin, and Oil Control	10	30	99	8.1

* $P < .05$ between group III and IV (control).
** $P < .01$ between group I and IV (control).

Corticosterone treatment, with or without gonadotropin, resulted in a significantly higher proportion of days ($P. < .01$) when parasites were detectable than was evident in the oil control group (Table 1). There was, however, little enhancement of the effect of corticosterone when corticosterone and gonadotropin alone was not significantly different than that found in the oil control group.

When the two groups receiving gonadotropin (II and III) are combined and compared with groups receiving no

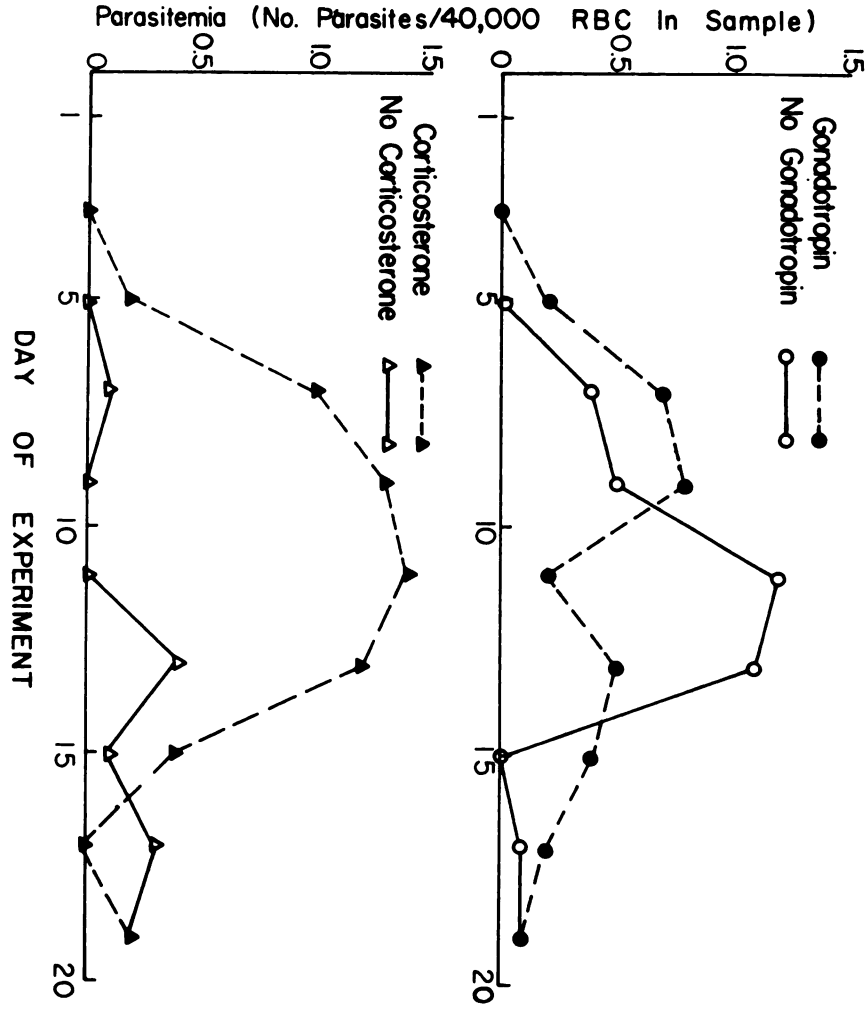

FIGURE 1. *The effect of gonadotropin and corticosterone on parasite populations in established infections. Groups II and III (see Table 1) received gonadotropin. Groups I and IV received no gonadotropin. Groups I and III received corticosterone, while groups II and IV received no corticosterone.*

gonadotropin (I and IV), it is apparent that gonadotropin had limited or no effect in elevating parasite populations (Table 1 and Fig. I). Comparison of groups receiving corticosterone (I and III) with groups receiving no corticosterone (II and IV) indicates that parasite population increases found in this study are related to the effect of corticosterone treatment (Table 1 and Fig. I).

In our earlier demonstration of corticosteroid induction of relapse, infections in experimental birds were made with whole blood transfers.[1] In the present study, infections result from natural mosquito transfer of sporozoites. A comparison between the corticosterone group in this study and the January corticosterone group in the previous study (Table 2) suggests that the method of infection did not affect the response of parasite populations to corticosteroid treatment.

TABLE 2. *A comparison between the effect of corticosterone on blood-induced infections (Applegate, 1970) and on sporozoite-induced infections.*

Parameter	Blood-induced (Jan 1968)	Sporozoite-induced (Dec 1969 - Jan 1970)
No. birds in sample	6	5
% birds relapsed	83.3	60.0
No. blood films made	56	40
% blood films with parasites	26.8	30.0
Maximum parasitemia in a bird	8/40,000 RBC	7/40,000 RBC
Highest mean parasitemia for sample	1.8/40,000 RBC	2.4/40,000 RBC

Discussion

Spring relapse has long been recognized in vivax malaria of humans. Coatney and Cooper[5] showed that this phenomenon resulted from a tendency for relapse to occur at a fixed time interval following infection. Since transmission occurred during the late summer and early fall, these relapses were concentrated in the spring. Our investigations with bird malaria, in which transmission occurred throughout the year, indicated that relapses occur in the spring regardless of the time the bird was infected.[1,2] These observations agree with the work of Chernin[6] on a related parasite, *Leucocytozoon*. Chernin artificially increased day length during the winter and succeeded in hastening the onset of reproductive behavior and *Leucocytozoon* relapse in ducks. It appears, therefore, that relapse in bird malaria is mediated by some physiological change in the host rather than being an intrinsic attribute of the parasite.

The most apparent physiological changes occurring in birds in the spring are those associated with reproductive activity. In one case,[8] male and female sex steroids were reported to stimulate relapse of *Haemoproteus* and *Leucocytozoon* in birds. Attempts of other investigators to induce relapse with exogenous sex steroids have failed.[3,7,10] In the present study the attempt to induce "spring" relapse by use of gonadotropin in a normally quiescent period was unsuccessful. Likewise, the attempt to potentiate the relapse-inducing activity of adrenal corticoids with gonadal stimulation was unsuccessful.

The physiological mechanism of spring relapse in bird malaria remains an unsolved problem. It appears, however, that if the solution lies in the birds' reproductive endocrinology, the mechanism is a more subtle one than has yet been tested.

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