EFFICACY OF ORAL SULFADIMETHOXINE AGAINST TWO GREGARINE PARASITES, *PROTOMAGALHAENSIA GRANULOSAE* AND *GREGARINA CUBENSIS* (APICOMPLEXA: EUGREGARINIDA), INFECTING THE DEATH'S HEAD COCKROACH, *BLABERUS DISCOIDALIS*

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ABSTRACT: Gregarines are common parasites of insects in culture, but no effective chemotherapeutic or prophylactic control protocol has been demonstrated. Sulfadimethoxine was administered in 5- and 7-day treatments to Death's Head cockroaches (*Blaberus discoidalis*) infected with *Gregarina cubensis* and *Protomagalhaensia granulosae* to test the efficacy of this sulfonamide against gregarine infection. Sulfadimethoxine significantly reduced the mean intensity of both *G. cubensis* and *P. granulosae*. Sulfadimethoxine treatment reduced gregarine intensity by 80% to 85% but had no significant effect on gametocyst production, suggesting that sulfonamide toxicity is directed primarily at sporozoites, trophozoites, and perhaps young gamonts. The possible use of sulfadimethoxine to produce gregarine-free insect cultures and the potential utility of gregarines as target organisms for screening pharmacologically active compounds for use against other intestinal apicomplexans are discussed.

Gregarines are probably the most taxonomically diverse and ubiquitous of the apicomplexan parasites. They are reported from a wide range of nonvertebrate hosts but are most commonly reported from arthropod and especially insect hosts. There is no recent review of gregarine pathology, but the literature is rife with examples ranging from reduction in host longevity or reduced host fecundity to epidemic mortality (e.g., Kulka and Corey, 1984; Jackson, 1985; De Montaigne and Maouloud, 1986; Zuk, 1987a, 1987b; Lipa and Triggiani, 1989; Purrini and Keil, 1989; Wu and Tesh, 1989; Munstermann and Wesson, 1990; Dougherty et al., 1991; Sulaiman, 1992; Sulaiman et al., 1993; Garcia et al., 1994; Pushkala and Muralirangan 1997, 1998; Iperti, 1999; Thomas et al., 1999; Johny et al., 2000). Control of gregarine infections is becoming increasingly important as the economic importance of arthropod monoculture increases.

Although the literature contains several examples of attempts to control gregarine infection in insectaria by sanitary methods (e.g., Jackson, 1985; Dougherty et al., 1991), only 1 previous study investigates the chemotherapeutic control of gregarines. Mourya et al. (1985) assessed the efficacy of metronidazole and sulfadimethoxine against *Ascogregarina culicis* (Eugregarinida: Lecudinidae) infecting larval *Aedes aegypti* (Diptera: Culicidae). Although metronidazole had no significant effect, high concentrations of sulfadimethoxine reduced the mean intensity in adult mosquitoes. Unfortunately, this effect was offset by dramatic increases in larval mortality. The study presented herein evaluates the efficacy of oral sulfadimethoxine (2,4-dimethoxy-6-sulfanilamideo-1,3-diazine) for chemotherapeutic control of 2 gregarine species, *Gregarina cubensis* and *Protomagalhaensia granulosae*, infecting the Death's Head cockroach, *Blaberus discoidalis*.

MATERIALS AND METHODS

A *B. discoidalis* colony was established using stock purchased from Carolina Biological Supply (Burlington, North Carolina). Initial survey of the colony indicated 100% prevalence of 2 gregarine species in the gut, *P. granulosae* and *G. cubensis*, both of which pass gametocysts, as well as 1 species of pinworm and 1 ciliate species in the rectum.

For each replication of the experiment, 3 treatment groups of 10

cockroaches each were maintained in individual plastic shoe boxes with wood-chip bedding. Each group was supplied with dog food and a water supply. One group was designated as a control group, 1 as a 5-day-treatment (D5) group, and the third as a 7-day–treatment (D7) group. Treatment groups were given an oral suspension of sulfadimethoxine (2,4-dimethoxy-6-sulfanilamideo-1,3-diazine) each day. Approximately 170 mg sulfadimethoxine per kilogram of body weight was administered to each test cockroach every day, whereas control roaches were fed a saline solution. There is no available dosage recommendation for sulfadimethoxine in arthropods; the 170-mg/kg dose used in this study is the standard sulfadimethoxine dosage approved in the United States for commercial livestock. To administer treatments, individual cockroaches were held upside down and fed a calibrated oral solution of sulfadimethoxine or saline by placing the dose on the cockroach's mandibles dropwise with a syringe. Cockroaches were allowed to completely imbibe each drop before subsequent drops were administered. The process continued until each roach consumed a full treatment dose. Half the control roaches were killed and examined for infection postmortem with the D5 group after 5 days of treatment. The remaining control roaches were killed and examined for infection postmortem with the D7 group after 7 days of treatment. The entire protocol was replicated 3 times.

Gregarine infections are often quite intense, making it difficult to count all the individual parasites in each host. Figures 1 and 2 demonstrate the intensity of a typical untreated infection in the central mesenteron. The gregarines infecting *B. discoidalis* are readily distinguished one from the other by differences in overall shape and size (Figs. 1, 2). *Protomagalhaensia granulosae* is proportionally much longer and thinner than the more massive *G. cubensis*. The comparative morphology of these gregarine species is discussed by Peregrine (1970). Both gregarine species exhibit site specificity in the cockroach gut, and this specificity was used to estimate gregarine intensity for each species. Major anatomical landmarks of the cockroach gut are marked in Figure 3. *Gregarina cubensis* populations consist primarily of immature forms located in the intestinal cecae, although mature associated gamonts of *G. cubensis* also occur in the upper mesenteron. In contrast, all developmental stages of *P. granulosae* reside exclusively in the posterior mesenteron. Gametocysts of both groups are found in the posterior mesenteron and rectum. During the postmortem examination, all individuals of *P. granulosae* were counted. All individual *G. cubensis* in 1 cecal pouch were counted as an index of immature *G. cubensis* intensity. (A pilot study of variation in cecal intensity found no significant difference in *G. cubensis* intensity among cecal pouches within individual roaches.) All individuals of *G. cubensis* in the ileum were counted as a measure of mature gamont intensity. Data were analyzed using a univariate ANOVA blocking on treatment and replication. Differences among treatment groups were identified using Student's 2-tailed *t*-test with Bonferroni's correction for multiple comparisons. All analyses were conducted using SPSS Base 10.0 (SAS Institute, Carey, North Carolina) with $\alpha = 0.05$.
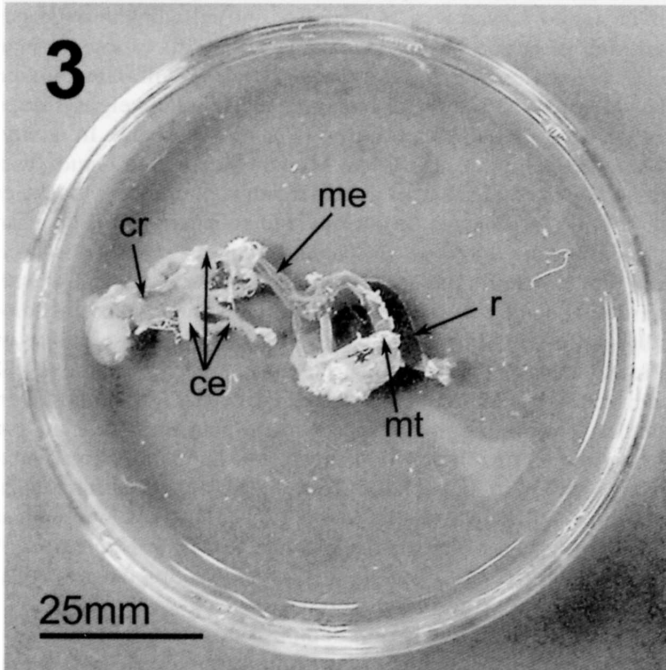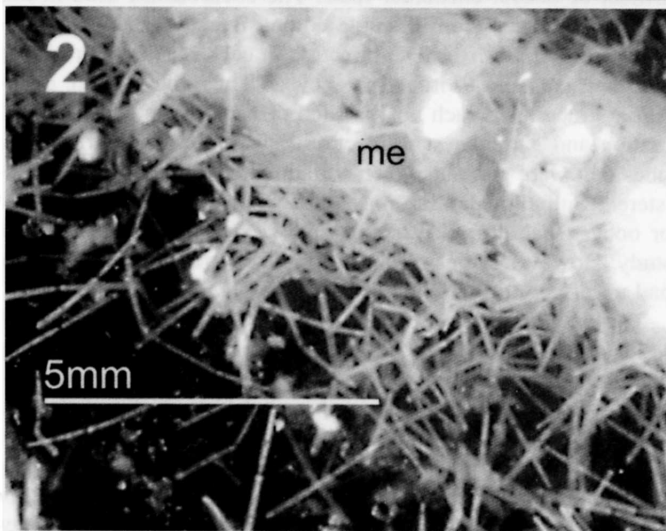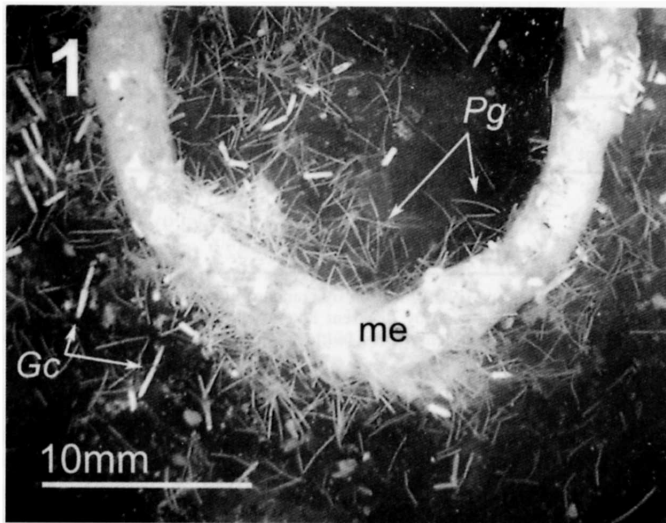
RESULTS

The effects of oral sulfadimethoxine treatment on gregarine intensity are summarized in Figure 4. The intensity of *P. granulosae* in control cockroaches was significantly higher than that of cockroaches receiving oral sulfadimethoxine (ANOVA, $F = 26.7$, df = 2, $P < 0.001$), although there was no significant difference in the intensity of *P. granulosae* among the 5- and 7-day–treatment groups (Table I). Similarly, the overall intensity of *G. cubensis* was lower in treatment groups. The intensity of *G. cubensis* in the cecae of control cockroaches was significantly higher than that of cockroaches receiving oral sulfadimethoxine (ANOVA, $F = 49.3$, df = 2, $P < 0.001$), although there was no significant difference in the intensity of cecal *G. cubensis* populations among the 5- and 7-day–treatment groups (Table I). The intensity of *G. cubensis* in the mesentera of control cockroaches was significantly higher than that of cockroaches receiving oral sulfadimethoxine (ANOVA, $F = 32.6$, df = 2, $P < 0.001$). Again, there was no significant difference in the intensity of mesenteric *G. cubensis* populations among the 5- and 7-day–treatment groups (Table I). In contrast to the intensity of trophic gregarine stages, there was no significant difference between the number of gametocysts found in the intestines of control cockroaches and of those receiving oral sulfadimethoxine (ANOVA, $F = 1.9$, df = 2, $P > 0.05$).

DISCUSSION

Changes in gregarine mean intensity (Fig. 4) demonstrate a reduction in the numbers of *P. granulosae* and *G. cubensis* in the groups treated with sulfadimethoxine. The lack of significant changes in gregarine mean intensity among the 5- and 7-day–treatment groups, and gametocyst production among control and treatment groups, indicates that sulfadimethoxine does not affect the gregarine population uniformly. The drug apparently acts within a developmental window, acting specifically against early ontogenic stages rather than against all endogenous stages of the gregarine life cycle. Such a pattern of ontogenic specificity would reduce the overall gregarine intensity by halting sporozoite and trophozoite development. Lack of a significant effect on gametocyst production reflects the normal completion of the life cycle by existing gregarine populations that had already passed through the developmental window in which sulfadimethoxine acts. These results are consistent with

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FIGURES 1–3.   Alimentary tract dissection of an untreated cockroach (*Blaberus discoidalis*) infected with the gregarines *P. granulosae* and *Gregarina cubensis*. **1.** Gamont associations of *P. granulosae* and *G. cubensis* in the central mesenteron, demonstrating the intensity of an untreated infection. The mesenteron has been slit, allowing gregarines to escape into the dissection arena. Associations of *P. granulosae* are relatively longer and thinner than the more massive gamonts of *G. cubensis*. **2.** Gamont associations of *P. granulosae* in the central mesenteron at increased magnification, providing details of morphology and demonstrating the dense packing of parasites in the untreated mesenteric lumen. **3.** Major anatomical landmarks of the alimentary tract of *B. discoidalis*. The intersection of cecal pouches and crop mark the anterior reach of the mesenteron, which extends to the basal attachment of the Malpighian tubules. *Gc*, *G. cubensis*; *Pg*, *P. granulosae*; ce, cecal pouches; cr, crop; me, mesenteron; mt, basal attachment of Malpighian tubules; r, rectum.
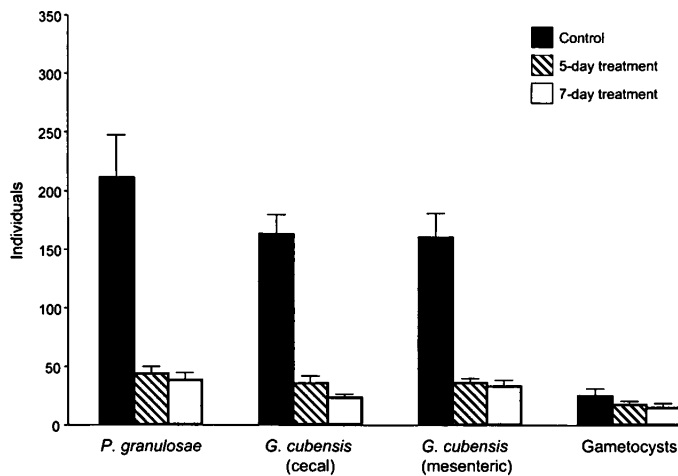
FIGURE 4.   Intensity of *Protomagalhaensia granulosae* and *Gregarina cubensis* in *Blaberus discoidalis* control, 5-day–treatment group, and 7-day–treatment group. Treatment groups received ca. 170 mg sulfadimethoxine per kilogram of body weight as oral suspension daily. Values presented are means with standard error.

TABLE I.   Intensity of *Protomagalhaensia granulosae*, *Gregarina cubensis*, and gregarine gametocysts in *Blaberus discoidalis* after 5- and 7-day treatment with 170 mg/kg oral sulfadimethoxine.*

Treatment	*P. granulosae*†	*G. cubensis* (cecal)	*G. cubensis* (mesenteric)	Gametocysts
Control	210.7 (36.6) a	162.5 (17.6) a	159.6 (20.6) a	24.5 (6.1) a
5 days	43.3 (5.1) b	35.2 (6.1) b	35.7 (3.9) b	17.1 (2.4) a
7 days	37.8 (5.2) b	23.8 (3.8) b	32.7 (5.4) b	14.5 (3.0) a

* Values are means (n = 30) followed by standard errors in parentheses.
† Treatment values followed by different letters within a single column are significantly different (Student's *t*-test with Bonferroni's correction for multiple comparison, $P < 0.01$).

what is known about the pharmacology of sulfadimethoxine and its mode of action against apicomplexan parasites.

Sulfadimethoxine belongs to a class of antimicrobial agents called sulfonamides that have been widely used in human and veterinary clinical practice since the 1940s (Cates, 1986). They display some apicomplexan toxicity and are commonly used for prophylactic control of coccidians in livestock and poultry (Levine, 1973; Cates, 1986; Chambers and Jawetz, 1998). The mechanism of action is similar in all sulfonamides, which ultimately control microbial infection by disrupting nucleic acid biosynthesis (for pharmacology, see Cates, 1986; Chambers and Jawetz, 1998). They display microbiostatic rather than microbiocidal activity (Chambers and Jawetz, 1998), i.e., sulfonamides do not directly kill microbes, but they halt the growth and reproduction of microbial cells.

The mean intensity trends presented in Figure 4 are consistent with the microbiostatic action that is specific to the early ontogenic stages. There were significant reductions of *P. granulosae* and *G. cubensis* populations over the treatment period, and no trophozoite or young gamont was observed in the 5- and 7-day–treatment cockroaches, indicating that pregamont infections (sporozoites, young trophozoites) were suppressed. Given the pharmacological mechanism of sulfadimethoxine, nucleic acid synthesis appears to be a critical developmental process in gregarine maturation from trophozoite to gamont. In contrast, there was no significant difference in gametocyst production over the treatment period, suggesting that late-stage gamonts are able to mature and form gametocysts without additional nucleic acid synthesis. Other studies have demonstrated the stage-specific nature of nucleic acid formation by apicomplexans (Newbold, 1982; Zhu et al., 1990; Gantt et al., 1998; Lang-Unnasch and Murphy, 1998), and the stage-specific action of sulfonamides against merogonic and schizogonic coccidian stages has been known for at least 30 yr (Levine, 1973). The data presented here suggest that sulfonamides have a similar gross action against gregarine populations in vivo, suppressing or preventing new infections without preventing the development and maturation of late-stage gamonts.

The lack of significant changes in gregarine mean intensity among the 5- and 7-day–treatment groups and gametocyst production among the control and treatment groups may also be an artifact of oocyst encounter rates. The cockroaches used in this study were taken from a single colony infected with *P. granulosae* and *G. cubensis*. The infection in each host is the result of constant exposure to infective oocysts. Thus, the parasite population structure in each host is such that relatively constant maturation and gametocyst production rates are expected of gregarines that were gamonts when sulfadimethoxine was first administered. Sulfadimethoxine may have some effect on gametocyst or oocyst viability, but these issues were not addressed in this study. However, Mourya et al. (1985) reported that sulfonamide had no effect on the viability of *Ascogregarina* sp. oocysts.

The results of this study differ significantly from those of Mourya et al. (1985), who observed up to 52% host mortality after treating mosquito larvae with sulfadimethoxine. In contrast, no experimental cockroach was lost to pharmacological toxicity in the present study. This difference is probably a result of differences in the treatment protocol. In the present study oral sulfadimethoxine was administered at label dosages for livestock, whereas Mourya et al. (1985) simply treated cultures of mosquito larvae at a rate of 0.5 mg sulfadimethoxine per milliliter of culture water. Mourya et al. (1985) exposed larvae to a constant environmental concentration of sulfadimethoxine that is at least 3 times the recommended tissue-weight dosage for the drug (500 mg/kg water vs. 170 mg/kg tissue). In reality, the tissue-weight dosage in the Mouyra et al. (1985) study was much higher. The substantial host death reported by Mourya et al. (1985) can probably be attributed to nephritic failure because of sulfonamide overdose. Sulfadimethoxine is readily absorbed into serous fluids, but its solubility decreases markedly with increasing acidity (Cates, 1986). Insects maintain a low pH distally within the Malpighian tubules, increasing the solubility of uric acid in the tubule lumen. Neutral or slightly alkaline pH levels are maintained at the proximal end of the Malpighian tubules, promoting precipitation of uric acid into the hind gut for subsequent excretion (Chapman, 1971). This pH-mediated excretory system exacerbates the precipitation of sulfonamides whose solubility is lowest at low pH (Cates, 1986). We suggest that high dosages of sulfadimethoxine lead to precipitation of the drug within the distal lumen of the Malpighian tubules, resulting in nephritic failure and death.

The study presented herein demonstrates the efficacy of sulfadimethoxine as a gregarinostat. Sulfadimethoxine appears to abort the development of gregarine sporozoites, trophozoites,

and early-stage gamonts without disrupting the ability of late-stage gamonts to form gametocysts. The viability of gametocysts formed in hosts treated with sulfadimethoxine is unknown. Sulfonamides may be harmful to insects at high dosage levels, but sulfadimethoxine reduced gregarine intensity by 80 to 85% in this study, so these drugs may be useful for control of gregarine infection in arthropod rearing systems. The observed efficacy of sulfadimethoxine coupled with its stage-specific microbiostatic action suggests that prophylactic control of gregarine infection in arthropod monoculture is possible if treatment is administered for a period of time extending beyond the life expectancy of a single gregarine cohort. In our laboratory colonies, we observe average cycle times for *P. granulosae* and *G. cubensis* of about 14 days, oocyst to oocyst. We hypothesize that sulfadimethoxine treatment for 14–21 days will eliminate young sporozoites, trophozoites, and young gamonts, allow mature gamonts to complete their life cycle and pass, and prevent the establishment of new infection. Subsequent sanitation of colony containers and bedding should result in a gregarine-free cockroach colony. At a minimum, periodic use of sulfadimethoxine should substantially reduce gregarine mean intensity in insect cultures.

On a more general scale, it has not escaped our attention that gregarine systems may have some potential utility as pharmacological screening tools. Gregarines appear to respond to sulfadimethoxine in a manner similar to that of coccidians. Insect–gregarine models are inexpensive relative to livestock–coccidian models and might be useful in screening pharmacologically active compounds for use against other intestinal apicomplexans, particularly coccidians.

ACKNOWLEDGMENTS

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