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Effect of Bacterial Lipopolysaccharide-induced Fever on the Humoral Response of New Zealand White Rabbits

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Tropisternus ellipticus (LeConte) is found from Oregon to central Iowa and east central Missouri, then south through Central America (Spangler, 1960). We report it for the first time from Arkansas. For our rarest *Tropisternus* species, we have seven collections of one specimen each, and each is from a different county (Fig. 6). All specimens are from Ozark streams and were taken during January, February, April. June, July and November. Huggins and Harp's (1983) identification of this species in Franklin County material was incorrect.

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THE EFFECT OF BACTERIAL LIPOPOLYSACCHARIDE-INDUCED FEVER ON THE HUMORAL RESPONSE OF NEW ZEALAND WHITE RABBITS

The response to microbial infection and microbial products is often an elevation of body temperature commonly termed fever. It seems likely that fever may be a beneficial host response providing a mechanism that aids survival from infections (Kluger *et al.*, 1975; Kluger and Vaughn, 1978). Kluger and Vaughn (1978) and Dinarello and Wolff (1978) noted that infected rabbits had a higher survival rate at moderate febrile levels; although, the reverse occurred at high body temperatures. Largely resulting from advertising, the American public is conditioned to routinely administer antipyretics during infections to suppress the fever response. Recently, Doran *et al.* (1989) reported that the healing time was prolonged in 37 children with chicken pox who received the antipyretic acetaminophen compared to 31 children receiving a placebo. Graham *et al.* (1990) also reported a negative effect against a Rhino virus infection when human volunteers receiving aspirin or acetaminophen produced lower titer antisera and shed virus for a longer time than those given a placebo.

The fever response can be initiated by exposing an animal to a variety of different chemical substances termed pyrogens including a number derived from infectious agents. Among the latter is bacterial lipopolysaccharide (LPS), a component of the outer membrane of Gram negative bacteria. This material produces dose-related fevers in humans and rabbits and has frequently been employed as a fever inducer for experimental purposes (Wolff, *et al.*, 1965; Wolff, 1973). A complicating factor in predicting and assessing the potential benefits of an LPS-induced fever is the fact that injection with this material may also trigger the release of ACTH and corticosterone which can result in immunosuppression (Moberg, 1971; Yasuda and Greer, 1978; Nakano *et al.*, 1987; Derijk *et al.*, 1991).

The current study was undertaken to examine the effects that fever and antipyretic fever suppression might have on the humoral response in rabbits. *Salmonella typhi* lipopolysaccharide (*Salmonella typhosa* No. L-6386, Sigma Chemical Co., St. Louis, MO) and acetaminophen were employed as the pyrogen and antipyretic respectively. Since LPS is itself antigenic, the antibody response to this substance was studied. However, to better assess the overall effects of fever and fever suppression, sheep erythrocytes were also employed as a second antigen.

Three groups each of seven New Zealand White rabbits (4.0 to 4.5 Kg) were employed in the study. Each animal was injected via a marginal ear vein on five separate days at 48 hr intervals (days 1-9) with 1.0 mL of a 10% suspension of sheep erythrocytes (S-RBC) in sterile saline. One group (S-RBC Only) received only the S-RBC while a second group (Fever Group) received an injection of a specified dose of LPS (8 ng/Kg on days one and three, 12 ng/Kg on day five, 15 ng/Kg on day seven, and 18 ng/Kg on day nine) along with the S-RBC. The increased dosage schedule was necessary because a resistance to the effects of LPS had been previously observed by day five in preliminary trials. A third group (Fever-Suppressed Group) was treated identically to the Fever Group except that acetaminophen was administered per OS in a dose of 100 mg/kg 15 minutes prior to each S-RBC/LPS injection. Immediately prior to each injection, a rabbit's base-line rectal temperature was measured and recorded. Temperatures were then recorded at one hour intervals post-injection for a period of four hours.

Sera were collected for analysis by intracardial bleedings using clot activating Vacutainer tubes (15 mL) equipped with a 20G 1.5" sterile needle. Pre-injection samples were taken one week prior to immunization while the post-injection samples were drawn six days after the last injection. All sera were clarified by centrifugation and kept frozen for later analysis.

Antibodies against the LPS were measured by performing tube agglutination reactions using a somatic (O) antigen prepared in our laboratory from *Salmonella typhi*. Antibodies to the sheep erythrocytes were measured by direct tube hemagglutination. Both the bacterial agglutination and hemagglutination reactions as well as the O antigen preparation were done according to the procedures of Garvey *et al.*, (1977).

The LPS injections produced an average peak rise in temperature of 1.2 C in the Fever Group while both the Fever-Suppressed and S-RBC Only Groups experienced an average peak rise in temperature of only 0.1 C. The fever was always apparent at the first measurement and in many cases was sustained throughout the four-hour period. In other instances the temperature had returned to normal after approximately 3 hours.

Bacterial agglutination reactions revealed a higher titer for post-infection antisera from rabbits in the Fever Group when compared to the Fever-Suppressed Group (Table 1.). The results of the sheep cell hemagglutinations (Table 2.) were similar to those of the bacterial agglutinations with the Fever Group once again providing the highest titer post-infection antisera. It was noted that the rabbits receiving only the sheep erythrocytes had an even lower titer than those in the Fever-Suppressed Group. Since both the latter groups of rabbits had the same peak rise in temperature it might be expected that their titers would also be nearly identical. The difference between the two groups was not great and might be nothing more than variation within a rather small sample of animals. The data from both serological studies suggest that the elevated body temperature of the rabbits did not act as a stressor to reduce the immune response but instead appeared to enhance antibody production. However, it is possible that the injected LPS itself simply had an adjuvant effect unrelated to the development of fever and the lower titers seen in the animals receiving acetaminophen might have been the result of some action by the drug other than antipyresis. Whatever the relationship might be between fever and the humoral response, it seems apparent that the administering of acetaminophen might interfere with an optimal antibody response.

Table 1. Effect of LPS-Induced Fever and Acetaminophen Fever Suppression.

Fever-suppressed Group	Fever Group	S-RBC Only
Pre/post-injection	Pre/post-injection	Pre/post-injection
<20/80	<20/160	<20/160
<20/160	<20/40	<20/80
<20/40	<20/160	<20/160
<20/160	<20/320	<20/160
<20/40	<20/80	<20/40
<20/160	<20/160	<20/80
<20/160	<20/80	<20/40
Mean: <20/114	<20/143	<20/103

Titer values are reciprocals of dilution factors.

Values of <20 represent the lowest dilution tested.

S-RBC Only represents injections with sheep erythrocytes but no LPS.

Table 2. Effect of LPS-Induced Fever and Acetaminophen Fever Suppression on Hemagglutination Reactions with Rabbit Sera.

Pre/post-injection	Pre/post-injection
<20/40	<20/160
<20/80	<20/40
<20/40	<20/320
<20/80	<20/160
<20/80	<20/80
<20/160	<20/80
<20/160	<20/160
Mean: <20/91	<20/143

Titer values are reciprocals of dilution factors.

Values of <20 represent the lowest dilution tested.

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