J. Thermal Biology, 1977, Vol. 2, pp. 79 to 81. Pergamon Press. Printed in Great Britain

FEVER IN THE FROG *HYLA CINEREA*

MATTHEW J. KLUGER
Department of Physiology, University of Michigan Medical School, Ann Arbor, Michigan 48109, U.S.A.

(*Received* 11 *September* 1976; *accepted* 23 *November* 1976)

Abstract—1. Injection of green tree frogs (*Hyla cinerea*) with dead *Aeromonas hydrophila* led to an average elevation in body temperature of over 2°C, with a latency of 2–4 hr.
2. Injection of green tree frogs with sterile pyrogen-free saline led to no change in body temperature.
3. Representative organisms from mammals, birds, reptiles, amphibians and fishes have now been shown to develop a fever in response to bacterial injections.

UNTIL a few years ago, it was generally assumed that mammals, and perhaps birds, were the only group of vertebrates to develop fevers in response to bacterial infections. In 1974, Vaughn *et al.* demonstrated that reptiles (the lizard *Dipsosaurus dorsalis*) develop a behavioral fever in response to inoculation with the Gram-negative bacterium *Aeromonas hydrophila*. Since that time it has been confirmed that birds develop a fever in response to bacterial infection (D'Alecy & Kluger, 1975). Reynolds *et al.* (1976) have shown that teleost fishes also develop behavioral fevers following injections of *A. hydrophila*.

In this study, green tree frogs (*Hyla cinerea*) were injected with bacteria and their thermal responses were recorded in order to determine whether amphibians can develop a behavioral fever. The results of these investigations indicate that these frogs will behaviorally elevate their body temperature following inoculation with *A. hydrophila*.

MATERIALS AND METHODS

Hyla cinerea were selected as our experimental animal since it has been reported in the literature that these frogs have a narrow preferred body temperature range (Brattstrom, 1970). The frogs, weighing 6–12 g, were obtained from a dealer in Tennessee and were housed in glass cages at an ambient temperature of *ca.* 22°C. Crickets and water were provided ad libitum. A 250 W heat lamp was suspended over each cage; this provided a continuous thermal gradient within each cage of from 22°C to over 35°C. Room lights were on a 12:12 photoperiod.

During experimentation, each frog was housed in a glass container (26 cm wide, 30 cm high, 49.5 cm long) covered with a wire mesh screen. A wetted pad on the floor of the container provided moisture. These containers were placed in a temperature and humidity controlled chamber at 20°C and 75% R.H. The photoperiod was 12:12. A 250 W heat lamp was suspended over one corner of the cage; this allowed each frog to regulate its body temperature between 20° and 35°C.

Temperature was recorded about every 30 s with copper–constantan thermocouples connected to a Honeywell Electronik 112 multipoint recorder. Body temperature of each frog was monitored by suturing a thermocouple junction sub-cutaneously in the mid-dorsal region. This temperature was found to be within 0.2°C of cloacal temperature.

Aeromonas hydrophila, a Gram-negative bacterium pathogenic to amphibians (the pathogen most commonly implicated in "red-leg" disease [Gibbs, 1963]), was grown on blood agar plates for 18 hr. The bacteria were then killed by suspending them in 70% ethyl alcohol for 1 hr. The dead bacteria were washed twice with sterile pyrogen-free saline (0.9% sodium chloride), centrifuged, and resuspended in saline. Confirmation that the bacteria were dead was made by plating 1 ml of the bacteria in saline on a blood agar plate and observing no growth. A concentration of 1×10^{10} dead bacteria/ml was prepared using Difco turbidity tubes as references.

Each frog was acclimated to the experimental chamber for 24 hr. The next day was designated as experimental day 1 and used as the control day. On the following day (experimental day 2) each frog was inoculated with 0.2 ml (sub-cutaneously) of either dead *A. hydrophila* (1×10^{10} bacteria/ml) or sterile pyrogen-free saline.

RESULTS

The mean hourly temperature (±S.E.) of 47 control frogs is shown in Fig. 1. The mean body temperature of these control frogs was about 25.5°C, with little circadian variation.

Injection of 31 frogs with *A. hydrophila* led to an average fever of over 2°C, with a latency of about 2–4 hr (Fig. 2). This elevation in body temperature was statistically significant ($P < 0.005$, Wilcoxon signed rank test). By 24 hr post-inoculation, body temperature had returned to control levels. Injection of 11 frogs with saline on experimental day 2 led to no elevation of body temperature. Without the 250 W heat lamp, frogs inoculated with bacteria were unable to elevate their body temperature, indicating that the fever was produced by behavioral selection of a warmer micro-climate, and not by endothermic means.

* Research supported by NSG GB 42749.

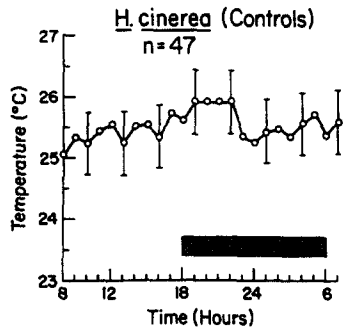

Fig. 1. Mean body temperature (±S.E.) of 47 green tree frogs (*H. cinerea*) over a 24 hr period. The cross-hatched bar indicates period of darkness.

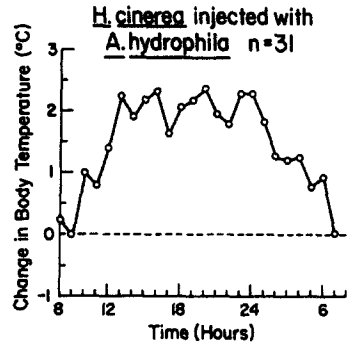

Fig. 2. Average changes in body temperature of green tree frogs (*H. cinerea*) in response to inoculation at 08.00 hr with dead *Aeromonas hydrophila* (0.2 ml of 1×10^{10} bacteria/ml).

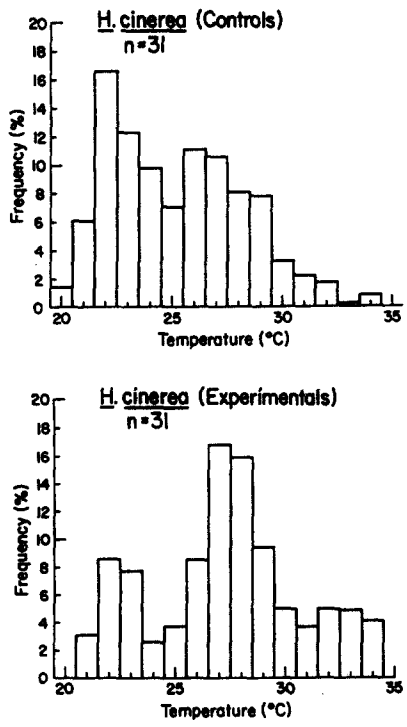

Fig. 3. Frequency distribution of body temperatures of 31 green tree frogs (*H. cinerea*). (A) Controls. (B) Experimentals (inoculated with *A. hydrophila*).

DISCUSSION

Green tree frogs develop a behavioral fever in response to inoculation with *A. hydrophila*. Unlike the results obtained by this laboratory on reptiles (Vaughn *et al.*, 1974; Bernheim & Kluger, 1976*a*, *b*) and birds (D'Alecy & Kluger, 1975), there is a great deal of individual variability in both the body temperature of the control frogs as well as in the response of these frogs to inoculation with bacteria. Variability in preferred body temperature appears to be a general characteristic of the Amphibia (Brattstrom, 1970) and might be attributable, in part, to competing drives related to salt and water regulation. Figure 3(a) and 3(b) shows the control and experimental data as a frequency–distribution plot. Whereas the mean body temperature of control frogs is about 25.5°C, this actually represents the average of a bi-modal distribution of body temperature with one peak at about 22°C and another at 26 or 27°C. Inoculation of these frogs with *A. hydrophila* leads to a dampening of the 22°C peak and a slight shift upwards of the second peak to 27 or 28°C. An analysis of the thermal responses of the 31 frogs inoculated with bacteria reveals that 15 had a mean temperature change (from 12.00 to 24.00 hr) of greater than 1.0°C (mean = 4.30°C ± 0.76 S.E.), 12 had essentially no change in body temperature (mean = 0.19°C ± 0.05 S.E.) and 4 had a mean 24 hr temperature decrease greater than −1.0 C (−2.67°C ± 0.17 S.E.). Overall, the rise in body temperature was statistically significant ($P < 0.005$, Wilcoxon signed rank test).

At the Third International Pharmacology of Thermoregulation Symposium, Reynolds & Covert (1976) presented data which indicated that bullfrog tadpoles (*Rana catesbeiana*) also developed behavioral fevers in response to inoculations of *A. hydrophila*. Based on their reported standard errors, there appears to be less individual variability in the febrile response in these larvae then observed in the adult tree frogs used in this study. It is possible that the larval stages of *H. cinerea* would also show less individual thermal variability. If, as has been demonstrated in lizards (Kluger *et al.*, 1975; Bernheim & Kluger, 1976*a*), a fever increases the percent survival of infected frogs, then one would expect to find little individual variation in the thermal responses during infection. If, however, a fever in response to infection is beneficial to the larval stage, but is of little adaptive value to the adult (i.e. a vestigial trait) then one might expect the type of variability seen in this study. This hypothesis is presently untested.

Acknowledgements—The author thanks Linda K. Vaughn for critically reading this mansucript, and Marlene A. Foster and Ricardo C. Alessio for their technical assistance.

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Key Word Index—Fever; frogs; *Hyla cinerea*; *Aeromonas hydrophila*; temperature regulation.