Pyrogens fail to produce fever in a cordylid lizard

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LABURN, H. P., D. MITCHELL, E. KENEDI, AND G. N. LOUW. *Pyrogens fail to produce fever in a cordylid lizard.* Am. J. Physiol. 241 (Regulatory Integrative Comp. Physiol. 10): R198–R202, 1981.—We investigated the effects on body temperature of the lizard *Cordylus cataphractus* of intracardiac injections of leucocyte pyrogen (LP) synthesized from rabbit blood and of killed *Aeromonas hydrophila*, a gram-negative bacterium reputed to be pathogenic in lizards. Lizards were placed in a photothermal gradient that allowed them to select a preferred body temperature following the injections. Neither injection of 0.5 ml rabbit LP nor of 4×10^9 organisms of *A. hydrophila* in 0.2 ml sterile saline caused body temperature of lizards to differ from that of control lizards injected with sterile saline. Following injection of these solutions in the lizards placed in a thermal gradient where ambient temperature ranged from 20–88°C, body temperature was maintained between 32 and 34°C. Pyrogens failed to elevate body temperature even when body temperature was elevated artificially to 36°C before injection. We conclude that *C. cataphractus* does not respond with fever to either rabbit LP or *A. hydrophila*. Fever may not be ubiquitous even among lizards.

leucocyte pyrogen; body temperature; *Aeromonas hydrophila*; *Cordylus cataphractus*

FEVER IN ECTOTHERMS was first observed in lizards (20) and has since been reported in amphibia, fish, and even some invertebrates (5–7, 12, 17–19). The existence of an ability to develop fever in such phylogenetically disparate animals has led some authors to suggest fever has an ancient evolutionary origin. Moreover, in at least some of the ectotherms, fever has survival value (8, 13), an observation of clinical importance if a similar advantage prevails in mammals.

The literature is remarkably devoid of reports of animals which fail to develop fever in response to pyrogen injection. Is fever really ubiquitous? We noticed that all work on fever in lizards appears to have been carried out using *Dipsosaurus dorsalis* and *Iguana iguana*, both iguanid lizards (2, 11). We, therefore, decided to investigate whether lizards of other families also had the ability to develop fever.

We describe here experiments in which we tested the responses of a cordylid lizard to pyrogens. *Cordylus cataphractus* inhabits arid regions of southern Africa and is well known for its sun-basking thermoregulatory behavior. The pyrogens selected for testing were those previously used by Kluger and his colleagues (2, 11) in studying iguanid lizards, namely the killed gram-negative bacterium *Aeromonas hydrophila* and rabbit leucocyte pyrogen.

Some of the results have been reported briefly at the Pecs Satellite Symposium on Thermal Physiology (15).

METHODS

Animals. Lizards (*C. cataphractus*) were trapped in the wild and maintained in the laboratory at an ambient temperature of 20–25°C. For several months before experimentation lizards were maintained on natural daylight cycles; light and heat were supplied by tungsten lamps that allowed the lizards to select their preferred body temperature. The lizards weighed between 33 and 60 g (mean 45 g; $n = 10$). New Zealand White rabbits of both sexes weighing between 2.5 and 3.0 kg were used.

Experimental chamber. During experiments, lizards were exposed in a photothermal gradient chamber with a sand base. Heat was supplied by tungsten lamps at one end of the chamber. The temperature at the cool end of the gradient was measured by exposed copper-constantan thermocouples and at the hot end by a copper-constantan thermocouple in a blackened copper tube of approximately the same dimensions as the lizards. Two gradient chambers were used. The first was an asbestos cement chamber with floor dimensions approximately 1.8 x 0.3 m. The temperature of the cool and warm ends of the chamber was 20–25°C and 80–88°C, respectively. The second chamber was a metal chamber of smaller dimensions, approximately 0.5 x 0.3 m, designed to fit inside an air-conditioned container. Here the cooler and warmer ends of the chamber were maintained at 35–37°C and 55–57°C, respectively.

Body temperature measurements. Lizard body temperature was measured by inserting a fine copper-constantan thermocouple (36 gauge) via the cloaca to a depth of approximately 30 mm. The thermocouple was secured to the lizard's tail with adhesive tape. Restriction of the lizard's general mobility within the chamber was small.

Rabbit body temperature was measured by inserting a copper-constantan thermocouple mounted in polyethylene tubing via the rectum to a depth of about 100 mm.

All thermocouple outputs were detected on a Bailey BAT4 thermometer at 15-min intervals. The thermocouples were calibrated in water against a standard mercury thermometer; precision of the calibrated thermocouples was better than 0.2°C.

Pyrogen injections. All injections in lizards were intracardiac and, except for the rabbit leucocyte pyrogen, were 0.2 ml. That the injectate indeed entered the heart was checked in a few lizards by injecting the same volume

of dye, killing the animals, and locating the dye at post-mortem. Intravenous 3-ml injections into rabbits were via an ear marginal vein.

Rabbit leucocyte pyrogen was prepared by incubating whole rabbit blood with purified endotoxin of *Salmonella typhosa* in a dose of 30 μg/100 ml of blood. Details of the technique have been described previously (4).

A. hydrophila was grown on blood-agar plates for 24 h before being killed by suspension in 70% ethyl alcohol. The organisms were then washed twice with sterile 0.9% sodium chloride, centrifuged, and resuspended. Concentrations of the killed bacteria in sterile 0.9% sodium chloride were adjusted using Burroughs-Wellcome turbidity tubes and checked by direct counting under the microscope. Injections into lizards consisted of 4×10^9 organisms in 0.2 ml of sterile saline. Injections into rabbits consisted of 4×10^9 organisms in 3 ml of sterile saline.

Control injections consisted of 0.2 ml of sterile saline in the case of the lizards and of 3 ml of sterile saline in the case of rabbits.

Experimental procedure. All experimental injections of the lizards were carried out at the same time of day, 1000 h. Lizards were kept 2 h in the experimental chambers before receiving any injections. Thereafter injections were made of either a pyrogen suspension or of sterile saline, and body temperature measurements were monitored for a further 7 h. In certain experiments temperature measurements were also made on the day following that of injection. Experiments on rabbits were carried out on conscious animals restrained in conventional stocks.

Statistical analysis of data. Data were subject to Student's *t* test and values of *P* less than or equal to 0.05 were considered to be significant.

RESULTS

Responses of lizards to injections of rabbit leucocyte pyrogen. Figure 1 shows the body temperature of lizards after injection of 0.5 ml of rabbit leucocyte pyrogen or of 0.5 ml sterile saline. Experiments were carried out in the chamber with the temperature range of 20–88°C; there were up to five lizards in the chamber at any one time. Each lizard received both leucocyte pyrogen and saline on separate days; the order of injection alternated from lizard to lizard.

Body temperatures of lizards receiving leucocyte pyrogen did not differ at any time from those of lizards receiving saline. Following both treatments, body temperatures stabilized on average between 32 and 34°C.

Responses of lizards to injections of A. hydrophila. Figure 2 shows the body temperature of lizards after injection of suspension of killed *A. hydrophila.* Conditions of the experiment were the same for injections of rabbit leucocyte pyrogen; control experiments consisted of the injection of 0.2 ml of sterile saline.

Body temperatures of all lizards rose slowly throughout the period of exposure, but at no time were temperatures of lizards receiving the pyrogen significantly different ($P > 0.05$, *t* test) from those of lizards receiving saline. Final body temperature following both treatments was about 32°C.

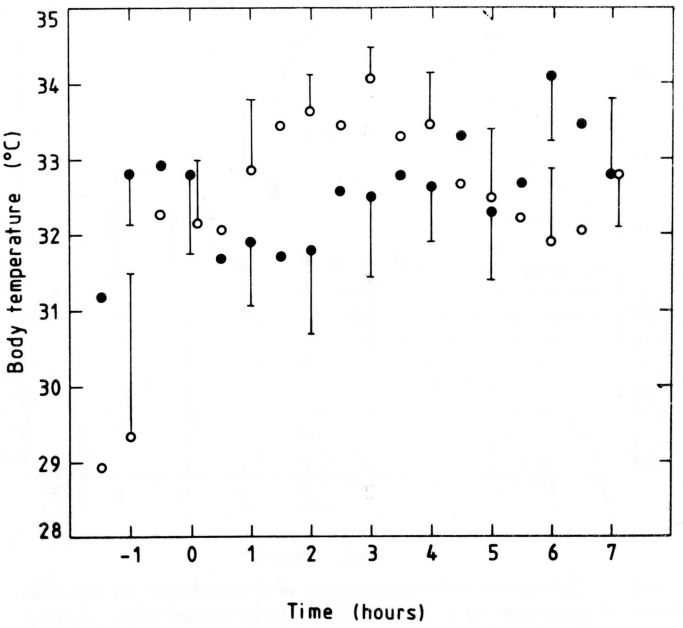

FIG. 1. Changes in body temperature of lizards following injection of 0.5 ml rabbit leucocyte pyrogen (LP) or of 0.5 ml sterile saline. Temperature of gradient was 20–88°C. Mean responses are shown for injections of rabbit LP (●) $n = 5$ or for injections of sterile saline (○) $n = 5$. One SEM is shown at hourly points. *Ordinate*: body temperature of lizards in °C. *Abscissa*: time (in hours) after injection (at zero).

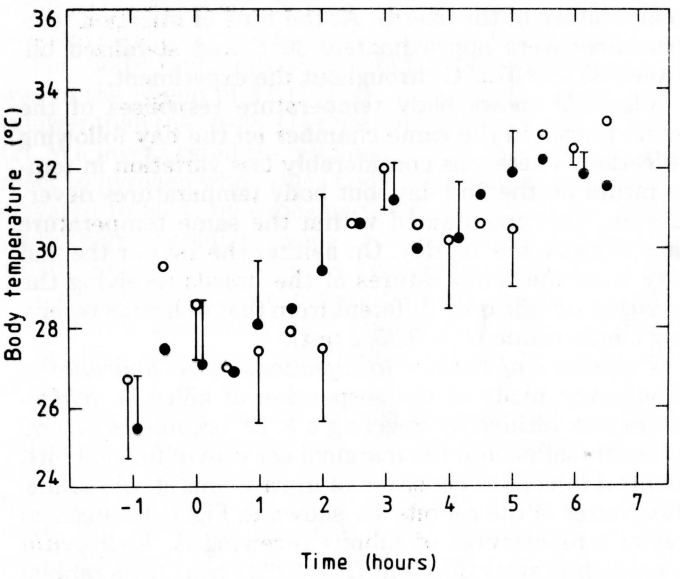

FIG. 2. Changes in body temperature of lizards following injection of 4×10^9 organisms of *A. hydrophila* in 0.2 ml sterile saline or of 0.2 ml of sterile saline. Temperature of gradient was 20–88°C. Mean responses are shown for injections of *A. hydrophila* (●) $n = 5$, or for injection of saline (○) $n = 5$. Additional details as in Fig. 1.

The results shown in Fig. 3 derived from experiments in which lizards were placed in the gradient chamber one at a time, rather than in groups. Body temperatures then stabilized at a somewhat lower level, on average 29–32°C. Once again there was no evidence that body temperature was different in the lizards following injection of the pyrogen.

In the next series of experiments, lizards were placed individually in the gradient chamber in which tempera-

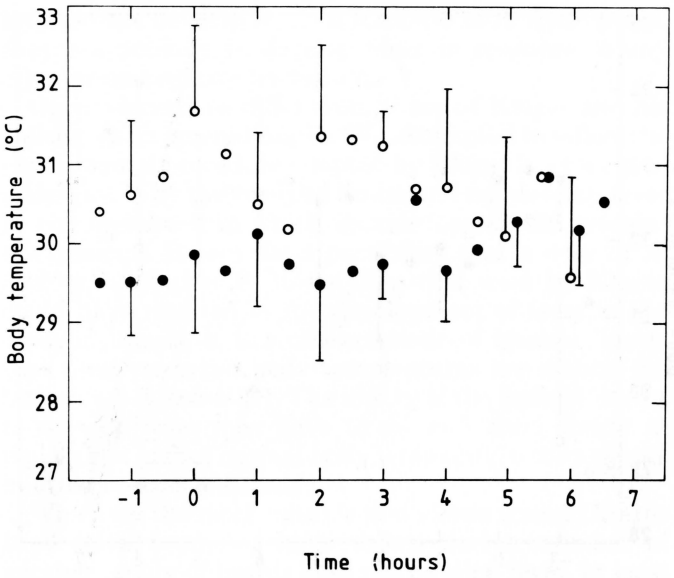

FIG. 3. Changes in body temperature of lizards following injection of 4×10^9 organisms of *A. hydrophila* in 0.2 ml sterile saline or of 0.2 ml of sterile saline. Temperature of gradient was 20–88°C and lizards were tested in gradient one at a time. Mean responses are shown for injections of *A. hydrophila* (●) $n = 6$ or for injection of saline (○) $n = 5$. Additional details as in Fig. 1.

ture ranged from 35 to 57°C. Injections were made of *A. hydrophila* or of sterile saline. Figure 4 shows the body temperature of the lizards. At the time of injection temperatures were approximately 36°C and stabilized between 35 and 37.5°C throughout the experiment.

Figure 5 shows body temperature responses of the same lizards in the same chamber on the day following injection. There was considerably less variation in temperature on the 2nd day, but body temperatures nevertheless were maintained within the same temperature ranges as on the 1st day. On neither the 1st nor the 2nd day were the temperatures of the lizards receiving the pyrogen significantly different from that of lizards receiving sterile saline ($P > 0.05$, t test).

Responses of rabbits to injections of A. hydrophila. The pyrogenicity of the suspension of killed *A. hydrophila* was verified by injecting 4×10^9 organisms in 3 ml of sterile saline into the marginal ear vein of four rabbits. Control injections consisted of intravenous sterile saline. Responses of the rabbits are shown in Fig. 6. Changes in rectal temperatures of rabbits receiving *A. hydrophila* were significantly different ($P < 0.05$, t test) from rabbits receiving saline from 30 to 120 min after injection.

DISCUSSION

Our results show that *C. cataphractus* did not develop fever in response to injections of rabbit leucocyte pyrogen or of endotoxin of *A. hydrophila*, an organism reported to be pathogenic in lizards (13). In all our experiments, body temperature changes in the lizards were the same following pyrogen injections as they were following saline injections.

That the lizards failed to develop fever in response to injections of rabbit leucocyte pyrogen was not due to a lack of pyrogenicity of the leucocyte pyrogen (4). It could be that the lizards failed to recognize the protein that constitutes rabbit leucocyte pyrogen, although Bernheim and Kluger (3) found that the desert iguana *D. dorsalis* does develop fever after injection of rabbit endogenous pyrogen. Whereas the idea that fever is of ancient evolutionary origin would be enhanced by the demonstration of cross-reactivity of endogenous pyrogens between phylogenetically different species, considerable species spec-

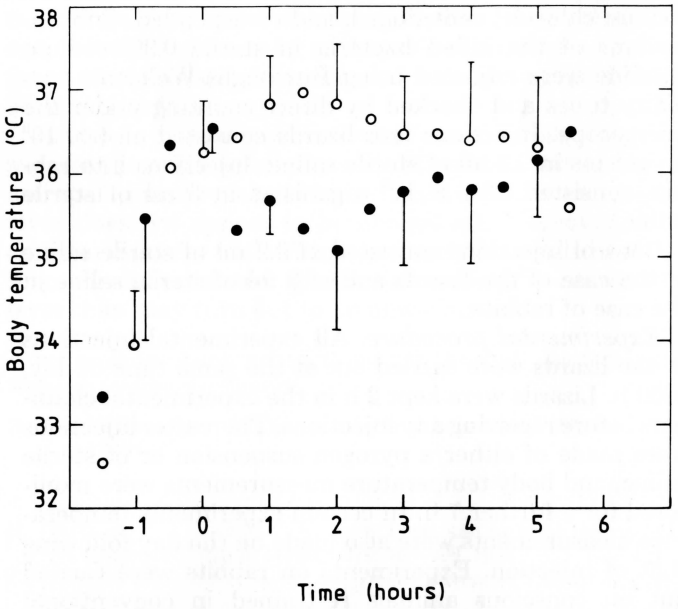

FIG. 4. Changes in body temperature of solitary lizards following injection of 4×10^9 organisms of *A. hydrophila* in 0.2 ml sterile saline or of 0.2 ml of sterile saline. Temperature of gradient was 35–57°C. Mean responses are shown for injections of *A. hydrophila* (●) $n = 5$ or for injections of saline (○) $n = 5$. Additional details in Fig. 1.

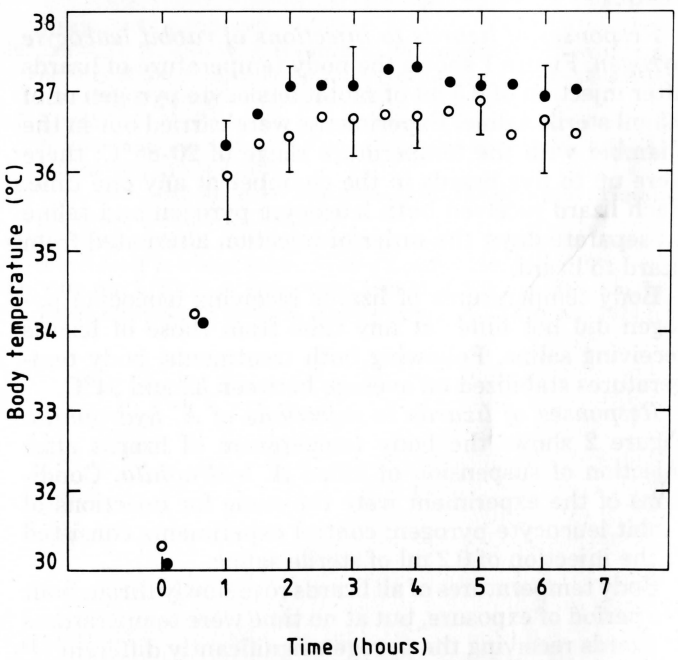

FIG. 5. Changes in body temperature of lizards on day following injection of either *A. hydrophila* (●) $n = 5$ or sterile saline (○) $n = 5$. Temperature of gradient was 35–57°C. All other details as in Fig. 4.

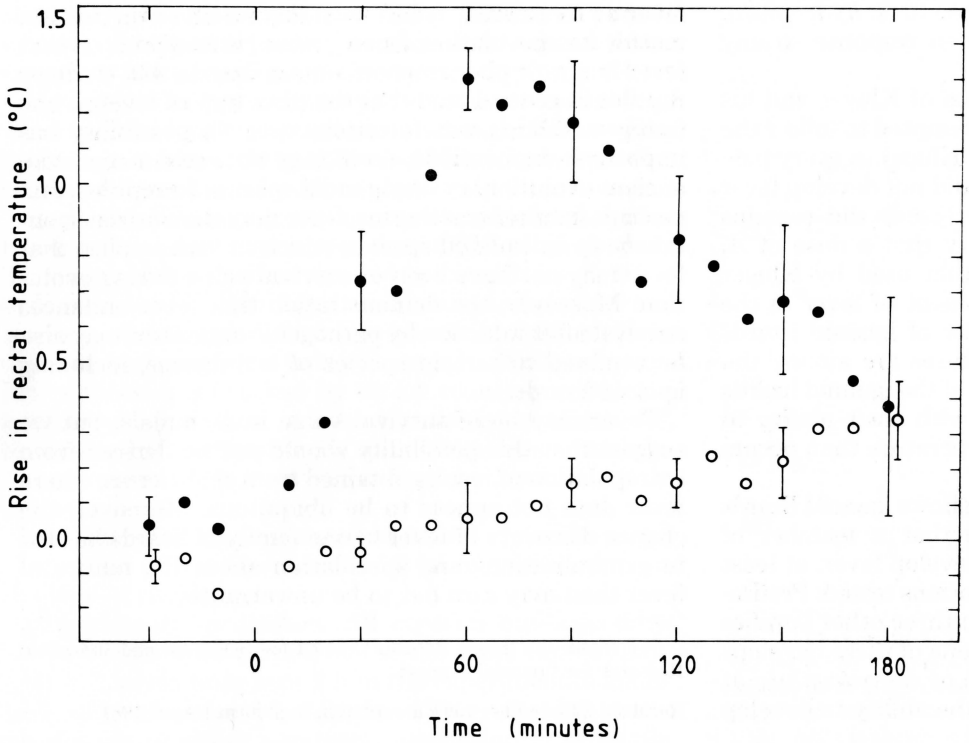

FIG. 6. Changes in rectal temperature of rabbits following injection of 4×10^9 organisms of *A. hydrophila* or of sterile saline. Mean responses are shown for injections of *A. hydrophila* (●) $n = 4$ or for injections of saline (○) $n = 5$. One SEM is shown every 30 min. *Ordinate*: change in rectal temperature from preinjection levels (zero on scale). *Abscissa*: time (in minutes) after injection (zero on scale).

ificity appears to exist even among species of mammals (4).

Kluger (13) and Bernheim and Kluger (2) found that two species of iguanid lizards, *D. dorsalis* and *I. iguana*, responded to the injection of *A. hydrophila* with a rise in body temperature brought about by selecting warmer environments. We prepared a suspension of killed *A. hydrophila* in a fashion identical to that of Kluger and his co-workers and injected it intracardically into *C. cataphractus* lizards free to select their thermal environment. The lizards did not select a warmer environment than they did when injected with saline (Fig. 2).

We attempted to discover why our lizards did not react to the injection of pyrogen, while the lizards studied by Kluger and his colleagues routinely did so. First, we established that the suspension of killed bacteria was indeed pyrogenic by injecting it intravenously into conscious rabbits. Injection of approximately the same absolute dose into rabbits, or about 1/16 of the dose per unit of body mass, caused a significant fever of rapid onset (Fig. 6). Thus the bacterial suspension was undoubtedly pyrogenic.

The second possibility that may have accounted for the difference between our results and those of Kluger lay in the nature of the behavior of the lizards. We observed *C. cataphractus* to display marked social facilitation (16); the lizards tended to cluster on top of and next to one another in all environments. It may have been that this social behavior masked any possible subtle differences in thermoregulatory behavior resulting from pyrogen injections. No mention was made of social facilitation in the case of the iguanid lizards. We therefore repeated the experiments exposing the lizards one at a time in the thermal gradient chamber. When exposed alone, the lizards selected somewhat lower body temperature (the decrease was not statistically significant) and attained this temperature more rapidly (Fig. 3), but there was still no evidence of a pyrexic response to the pyrogen.

The body temperature selected by *C. cataphractus* was in the region of 32°C (Figs. 1–3). This temperature is lower than the 36–38°C preferred by the iguanid lizards (2). We investigated the possibility that the lizards only react to pyrogen when their body temperature is already elevated to 36°C by exposing them, one at a time, to a photothermal gradient inside an air-conditioned chamber where the air temperature was controlled at 35–57°C. In this chamber, the lowest body temperature the lizards could attain was about 36°C. Injection of pyrogen failed to produce any significant change in body temperature (Fig. 4).

Finally, Kluger and his colleagues found that the elevation of body temperature following injection of *A. hydrophila* into iguanid lizards was greater on the day following the injections than on the day of the injection itself. We therefore recorded body temperatures of the cordylid lizards in the hot photothermal gradient on the day after injection. There was less variation on the 2nd day, and again there was no difference between those lizards receiving pyrogen and those receiving saline (Fig. 5).

All our results point to the conclusion that neither rabbit leucocyte pyrogen nor the pyrogenic suspension of killed *A. hydrophila* bacteria have any effect on the body temperature of *C. cataphractus* lizards. *A. hydrophila* is a gram-negative bacterium (10), which happens to be pathogenic in lizards. As is probably the case with all gram-negative bacteria, the pyrogenicity arises from the lipid moiety of the lipopolysaccharide of the cell walls (9). The lipid moiety, lipid A, is common to most, if not all, pyrogenic lipopolysaccharides. Thus, because the liz-

ards did not develop fever in response to *A. hydrophila*, they are unlikely to develop fever in response to any other gram-negative bacterium.

Our observations differ from those of Kluger and his colleagues on iguanid lizards. We attempted to follow the experimental procedure adopted by Kluger in every possible way. Why the cordylid lizards did not develop fever in circumstances in which iguanid lizards did remains unexplained. It remains a possibility that a dose of *A. hydrophila* higher or lower than that used by Kluger could have resulted in the development of fever in the cordylid lizards. It is a characteristic of iguanid lizards that their preferred body temperatures are among the highest of all lizards (1). The ability of the iguanid lizards to develop fever may have to do with their ability to maintain a higher normal body temperature than occurs in other species of lizards.

Whatever the mechanism is that allows iguanid lizards to develop fever, we have shown that a member of another family of lizards does not develop fever, at least in response to the two pyrogenic solutions tested. Preliminary observations we have made on three other families of lizards have demonstrated that none of these develops fever either in response to injection of *A. hydrophila* at the dose used by Kluger. Whether the ability to develop fever never existed, or has been lost, in these species is a matter for speculation. In any event, it may well be that fever is a rare phenomenon among lizards, and perhaps reptiles in general, and that the discovery of fever in one family of lizards was fortuitous. Such a possibility has important implications; fever may not have a common, ancient evolutionary origin in all species. In reptiles, and perhaps in other ectotherms, fever may have arisen spontaneously in isolated species, which in turn implies that fever may not have been of survival value during evolution. Moreover, the demonstration that fever enhances survival after infection by pathogenic organisms may also be confined to certain species of ectotherms, including iguanid lizards.

Fever may be of survival value in mammals, but we suggest that this possibility should not be derived from extrapolation of results obtained from ectotherms, where fever does not appear to be ubiquitous. Moreover, the chance discovery of fever in one family of lizards has led to generalizations and speculation about the nature of fever than may turn out to be unwarranted.

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