male solo of great calls with subdued climax, rallentando. We have heard the male's hoots between great calls early in the bout.

The hoolock's territorial song includes an accelerated passage of alternating high and low notes reminiscent of the siamang, except that the bass line gradually ascends. It is an elaborate duet (*4*), yet we heard a single female at the Calcutta zoo render the entire score as a solo. Tilson, observing in Assam, found that the preliminary high note is the male's signal which precipitates a rendition of the great call by his mate.

The Kloss gibbon's great call is probably the finest music uttered by a wild land mammal. Following the magnificent central trill is a slow, stepwise descent in a low register. (The soft first and last low-pitched notes are barely visible on the sonogram.) The fully elaborated predawn phrase of the male includes a trill (*3*), not shown; we heard these lovely sounds at 4 a.m. on a moonlit night from Tenaza's camp on South Pagai.

The pileated gibbon's territorial song is a duet of the pair plus a male coda, whereas that of Mueller's gibbon is separated into male predawn and female morning solos. The great call, practically identical in these two taxa, illustrates "the peculiar bubbling noise they make" (*5*).

Individual variation and to some extent population dialects are responsible for the differences in length and number of climactic notes of the otherwise identical great calls of agile and lar gibbons (Fig. 1, lower six songs). The males indulge in predawn choruses, which they can also elaborate at other times of the day. They provide mandatory fillers between great calls, plus a distinctive coda thereto: "who-hah" in the agile gibbon, hoots and lugubrious quavering notes in the lar.

Gibbon vocalizations, well correlated with coloration (*6*), point toward the existence of nine species of *Hylobates*, provided that no substantial breakdown of reproductive isolation will be found at the contacts of *H. agilis* with *H. lar* in the vicinity of Lake Toba, Sumatra, and at the Thai-Malaysia frontier and between *H. agilis* and *H. muelleri* on Borneo. Finding this proof is complicated by deforestation at Lake Toba, terrorism and banditry at the Thai-Malaysia border, and the fact that on Borneo the two taxa of gibbons appear to have identical coloration.

In conclusion, the faithfulness of each species to its prescribed musical score, together with pronounced sexual divocalism, make the voices of gibbons seem a powerful guide to the isolation of species by appropriate pairing and restriction to territories. That the selective forces put a higher reward upon differentiation of the male's voice than of the female's is evident from those taxa distinguished vocally by only the male's part of the territorial song.

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### References and Notes

1. Successfully used for *Cercopithecus* by T. Struhsaker, in *Old World Monkeys*, J. Napier and P. Napier, Eds. (Academic Press, New York, 1970), pp. 365–444.
2. J. Marshall, B. Ross, S. Chantarojvong, *J. Mammal.* **53**, 479 (1972).
3. R. Tenaza, *Z. Tierpsychol.*, in press.
4. Tenaza informs us that this observation is included in his detailed analysis of all gibbon vocalizations together with their social and evolutionary significance, which will appear as P. Marler and R. Tenaza, in *How Animals Communicate*, T. A. Seboek, Ed. (Indiana Univ. Press, Bloomington, in press).
5. C. Hose, *Mammals of Borneo* (Abbott, London, 1893), p. 60.
6. J. Marshall, Jr., and E. Marshall, *Mammal. Chromosomes Newsl.* **16**, 30 (1975).
7. We thank B. Galdikas-Brindamour, D. Holmes, P. Napier, B. Ross, K. Scriven, and R. Tilson for tape recordings which include songs used as audiospectrograms; D. Chivers, Greysolynne Fox, J. Romer, C. Wilson, and W. Wilson for additional tape recordings; Mr. Vandee Nongngork for assistance in the field; P. Winter, W. Brockelman, B. Chiarelli, D. Chivers, J. Delacour, A. Eudey, J. Fooden, P. Gittins, C. Groves, E. Mayr, J. McNeely, Lord Medway, P. Rand, R. Ronkin, B. Ross, R. Tenaza, R. Tilson, C. Wilson, and W. Wilson for information and comments on this report. We thank the authorities in charge and our colleagues for hospitality at the forest areas visited: Royal Thai Forest, Irrigation, Power, and Public Welfare Departments; Maxwell's Hill Station, Taiping; Office of the Chief Game Warden, Sandakan; Forest Department, North Sumatra and West Java; Museum Zoologicum Bogoriense and Herbarium Bogoriense; Messrs. Boeadi and G. Hambali (Java), R. Brindamour (Pangkalanbun), B. Smith (Sandakan), and R. Tenaza (Pagai). J.T.M. thanks SEATO Medical Research Laboratory, Bangkok, for travel within Thailand and photography.

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## Fever: Effect of Drug-Induced Antipyresis on Survival

Abstract. *To determine whether the prevention of fever affects the survival of an animal infected with pathogenic bacteria, lizards (*Dipsosaurus dorsalis*) were infected with live* Aeromonas hydrophila *and received varying doses of sodium salicylate, an antipyretic drug. Twelve lizards received identical injections of bacteria along with a nontoxic dose of sodium salicylate; five animals increased their mean body temperature at least 0.6°C and survived the week, whereas seven did not develop a fever and died within 3 days. These data indicate that in these lizards the prevention of fever by use of an antipyretic drug such as sodium salicylate increases the mortality rate from bacterial infection.*

The desert iguana (*Dipsosaurus dorsalis*) develops a fever in response to infection with *Aeromonas hydrophila* (*1*), a gram-negative bacterium pathogenic to reptiles and amphibians. This fever results from the lizard's selection of a warmer microenvironment. Because the febrile response is similar in reptiles, birds, and mammals, and because the body temperature of an ectotherm, or behavioral thermoregulator, such as the desert iguana can be easily controlled at either the normothermic or febrile level by simply adjusting ambient temperature, *D. dorsalis* was proposed as a suitable animal model for study of the role of fever in disease (*2*). Lizards were infected with *A. hydrophila* and then placed in constant temperature chambers adjusted to between 34° and 42°C. At temperatures of 40° and 42°C (corresponding to low and high fever, respectively), the mortality of lizards attributable to infection with *A. hydrophila* was 33 and 0 percent, respectively. The mortality at 38°C (the normal body temperature of afebrile or uninfected lizards) was 75 percent. These data indicated that an elevation in body temperature led to a significant reduction in host mortality (*2*).

In mammals, fever is often reduced by administering an antipyretic drug such as sodium salicylate (*3*). This drug produces effective antipyresis in reptiles and birds inoculated with dead bacteria (*4, 5*). However, the effects of drug-induced antipyresis on the survival of mammals, birds, or reptiles infected with pathogenic bacteria have not been determined. Because of the results described above (*2*), we suspected that the administration of a dose of sodium salicylate sufficient to produce an attenuation of fever in *D. dorsalis* would lead to a significant increase in host mortality. We report here that whereas the development of fever in *D. dorsalis* results in low mortality (less than 10 percent), suppression of fever by an injection of a nontoxic dose of sodium salicylate results in substantial mortality (100 percent in these studies).

Lizards (*D. dorsalis*) weighing 25 to 45 g (Hermosa Reptile Farm, Hermosa, California) were housed at an ambient temperature of 22° to 24°C in circular cages and had free access to mealworms, lettuce, and water. The cages were kept on a photoperiod of 12 hours light and 12

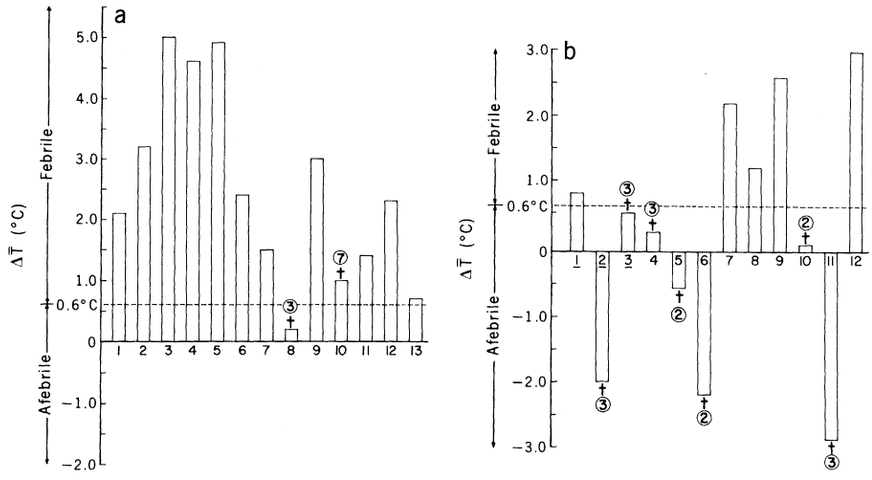


Fig. 1. (a) Average change in mean daily body temperature (0900 to 1800) for 2 days after inoculation of 13 desert iguanas with live *A. hydrophila*. Lizards that died are indicated by a cross. The number above each cross is the day after inoculation that the animal died. The dashed line at 0.6°C indicates the minimum increase in body temperature considered a fever (*5*). (b). Same as (a) except lizards were also injected with 4 mg of sodium salicylate. All animals with increases in body temperature greater than 0.6°C survived, whereas all others died.

hours dark (LD 12 : 12); a small area was heated to sand temperature of higher than 50°C by a 250-watt heat lamp that was also on the 12 : 12 cycle.

*Aeromonas hydrophila* was grown at 37°C on blood agar plates for 24 hours. The bacteria were suspended in sterile pyrogen-free saline (0.9 percent sodium choride), washed twice, centrifuged, and then resuspended in saline. The bacteria were diluted with saline to a concentration of $1 \times 10^{10}$ per milliliter as determined by comparing turbidity to McFarland barium sulfate standards. The dose of bacteria ($5 \times 10^9$ per lizard) had previously produced 75 percent mortality in lizards artificially held at 38°C (*2*). Sodium salicylate solutions (20 mg/ml) were prepared by dissolving sodium salicylate (Fisher Scientific Co.) in sterile pyrogen-free 0.9 percent saline. The solutions were then passed through a 0.2-$\mu$m Gelman Metricel filter to remove any contaminating pyrogens and plated on blood agar to test for sterility.

Experiments were performed in two chambers (1.4 by 1.2 m) covered on the bottom with about 15 cm of sand. These chambers were placed in a temperature-controlled room on an LD 12 : 12 photoperiod (light from 0600 to 1800). The ambient temperature was 30°C during the day, and 12°C at night. Suspended above the chamber were three pairs of heat lamps timed as follows: pair A, on from 0600 to 1000; pair B, on from 0900 to 1500; pair C, on from 1000 to 1800. As a result of this cycling, the lizards were forced to move to thermoregulate. The light intensity during the day approximated 770 lu/m$^2$ in the center of the chamber at floor level. Temperatures beneath the operating heat lamps were 50° to 55°C, while in other areas the temperatures approached ambient (30°C during the day). A copper-constantan thermocouple covered with polyethylene tubing was placed about 3 cm into each lizard's cloaca and taped to its tail. Each lizard was allowed to move freely throughout the chamber. Thermocouples were connected to a Honeywell Electronik 112 multipoint temperature recorder that recorded the temperature (± 0.1°C) of each lizard about every 30 seconds.

For each experiment lizards were allowed 1 day to acclimate to the chamber. Control data were collected the following day, and on the next day lizards were injected in the dorsal lymphatic space with live *A. hydrophila* (0.5 ml). For those experiments in which sodium salicylate was used 0.2 ml was injected in the coelom at 0900 and 1300 on days 3 and 4.

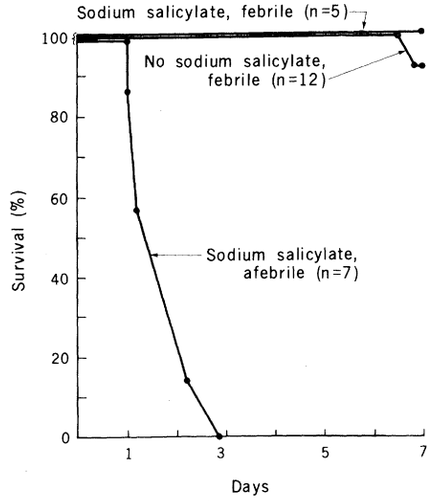


Fig. 2. Percentage survival of lizards inoculated with live *A. hydrophila* with and without sodium salicylate.

Four experiments were performed. In experiment A, intended to characterize the responses of lizards to infection with live bacteria without the administration of an antipyretic drug, 13 lizards were placed in the simulated natural environment and were injected with live *A. hydrophila* alone. Experiment B was designed to determine whether the injection itself had an effect on the thermal responses or survival of lizards; six lizards were injected with 0.5 ml of sterile pyrogen-free saline into the dorsal lymphatic space (controls). In experiment C, in which the effects of drug-induced antipyresis on the survival of infected lizards were determined, 12 lizards were injected with live bacteria and sodium salicylate. Experiment D was designed to determine whether the dose of sodium salicylate used in experiment C was toxic. Eight lizards were injected with bacteria and sodium salicylate and placed in glass containers inside a constant temperature chamber; their body temperatures were maintained at the febrile level by adjusting the chamber temperature to about 41°C during the day (the average temperature selected by febrile lizards in the simulated natural environment) and to 12°C at night (again, as in the simulated natural environment).

Mean hourly and daily body temperatures were calculated by taking the integrated means. Standard errors were calculated for each hour and each day. The data selected for analyses were from 0900 (when lizards were injected) to 1800. After 1800 the heat lamps went off and room temperature fell to 12°C, simulating a desert night. At night the lizard's body temperature was the same as the mean ambient temperature of 12°C.

The lizards injected with live bacteria but no sodium salicylate (experiment A) developed a fever averaging 2.3°C over a 5-day period (mean body temperature, 40.6°C) (*5*). By day 6, body temperatures returned to normal. One of the lizards did not develop a fever and died on day 3, while one lizard that did develop a fever died on day 7 (Fig. 1a). [We define fever as an increase in body temperature of 0.6°C or greater, in agreement with the *United States Pharmacopeia* definition of fever in rabbits (*6*).] Of those lizards developing a fever, 92 percent survived at the termination of the experiment, a value in agreement with that found earlier (*2*). These data also demonstrate that an elevation in body temperature need not be continuous since the lizard's body temperatures were lowered to 12°C at

night. The six lizards injected with saline alone (experiment B) did not develop any fever and all survived.

Five of the 12 lizards in experiment C developed a fever within 48 hours after injection with live bacteria and sodium salicylate. All five febrile lizards survived, while the seven afebrile lizards died (Fig. 1b). Although the sample sizes are small, these differences are statistically significant ($P < .01$, chi-square test). These data for experiments A and C are summarized in Fig. 2. In experiment D, only one of eight lizards died, which indicates that the dose of sodium salicylate used in these experiments was not toxic.

These data indicate that the administration of sodium salicylate to lizards with bacterial infections is harmful when it results in reduction of body temperature to an afebrile level. When sodium salicylate failed to produce antipyresis, the survival of infected lizards was not affected. It is not known why 5 of the 12 lizards receiving sodium salicylate developed a fever. The dose of sodium salicylate was kept low in order to minimize the toxic effects of this drug (*7*). The most likely explanation for the different responses to the salicylate is individual variability—that is, the dosage is probably on the ascending side of the dose response curve (*5*). In addition, initial results from our laboratory indicate that sodium salicylate is not 100 percent effective in preventing fever in mammals infected with live bacteria.

It is not known whether the results concerning the adaptive value of fever in reptiles can be extrapolated to the higher vertebrates, including man. We have shown that the characteristics of fever in the higher vertebrates (reptiles, birds, and mammals) are similar (*1, 4, 5*). For example, all three classes of vertebrates contain individuals that develop fever in response to injection with dead bacteria (containing endotoxin) or to infection with live bacteria. In all three classes, sodium salicylate is an effective antipyretic drug. Since the characteristics of fever are similar, it is tempting to suggest that the febrile mechanism had a common origin. If this is the case, we suspect that the function of fever in birds and mammals is similar to that in reptiles; that is, fever has evolved as a defense mechanism which substantially increases the likelihood of the infected host surviving that infection.

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**References and Notes**

1. L. K. Vaughn, H. A. Bernheim, M. J. Kluger, *Nature (London)* **252**, 473 (1974).
2. M. J. Kluger, D. H. Ringler, M. R. Anver, *Science* **188**, 166 (1975).
3. L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics* (Macmillan, New York, ed. 5, 1975), p. 1704.
4. H. A. Bernheim and M. J. Kluger, *Physiologist* **18**, 141 (1975); L. G. D'Alecy and M. J. Kluger, *J. Physiol. (London)* **253**, 223 (1975).
5. H. A. Bernheim and M. J. Kluger, *Am. J. Physiol.*, in press.
6. *United States Pharmacopeia* (Mack, Easton, Pa., ed. 19, 1975), p. 613.
7. In an initial study the dosage of sodium salicylate per lizard was 0.2 ml of a 37.5 mg/ml solution. This higher dose of salicylate prevented fever in only five of eight lizards (63 percent); this indicates that even at higher doses, sodium salicylate does not prevent fever in 100 percent of infected animals. Control lizards were injected with bacteria and this high dose of salicylate, and then maintained at a febrile body temperature by artificial means. Those injected with the high dose of salicylate had a statistically higher mortality (7 of 12 or 58 percent) than did controls injected with 0.2 ml of a 20 mg/ml solution as reported in the text (1 of 8 or 13 percent died; $P < .04$, chi-square test); this indicates that the high dose of salicylate is toxic.
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## Herbicide (2,4-D) Increases Insect and Pathogen Pests on Corn

Abstract. *Corn leaf aphids, European corn borers, and southern corn leaf blight were more abundant on corn exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide than they were on unexposed corn. Protein levels were higher in corn plants that were exposed to several dosages of 2,4-D, and this may have favored the growth of pests.*

Since 1945, increased losses due to attack of insects and pathogens have been reported for crops in spite of greater efforts in pest control (*1*). How much if any of this increased loss caused by pests is due to the ecological and biochemical impact of herbicides on crops is unknown, but in a number of instances herbicides have been reported to increase pest problems on various crops (*2*). Our laboratory and field tests were designed to determine what influence the use of 2,4-dichlorophenoxyacetic acid (2,4-D) has on the susceptibility of grain corn to the European corn borer (*Ostrinia nubilalis*), corn leaf aphid, and the southern corn leaf blight (*Helminthosporium maidis*).

In 1973 a preliminary study was made of the impact of 2,4-D (triethanolamine salt) herbicide on corn leaf aphid and European corn borer populations in the corn variety Pennsylvania 290. The three treatments of 2,4-D per hectare were (i) untreated (control), (ii) 0.14 kg, and (iii) 0.55 kg (normal use). The herbicide spray was directed at the base of knee-high corn plants and toward any weeds, and all plots were cultivated for weed control. Aphid counts were made on 60 ears of corn selected systematically from each of these plots during late September. The number of aphids, following the three treatments, were (i) 618, (ii) 1388, and (iii) 1679. Corn borer infestations were measured in late August, and the percentages of plants in these plots that were infested with corn borer larvae were 16 percent after (i), 24 after treatment (ii), and 28 after treatment (iii).

Table 1. Mean pupal weight and egg production of moths reared from corn borer larvae raised on hybrid corn OH 51A × B8 treated with four dosages of 2,4-D.

| Dosages of 2,4-D (ppm) | Mean pupal weight (mg)* | Mean number of egg masses per female |
|---|---|---|
| 0 | 92.87 c | 18.7 |
| 5 | 98.51 b | 26.0 |
| 20 | 113.43 a | 25.5 |
| 80 | 103.01 b | 32.5 |
| 320 | 91.63 c | 19.0 |

*Significant differences at 0.05 level (Duncan's multiple range test) indicated by letter differences.

More extensive field tests were made in 1974 on three row plots (70 to 90 plants) 2½ by 7 m in size. Four treatments (i) untreated (control), (ii) 0.14 kg of 2,4-D per hectare, (iii) 0.55 kg of 2,4-D per hectare (normal use dosage), and (iv) 4.4 kg of 2,4-D per hectare were used; techniques were the same as in the 1973 tests. Aphid counts made on the tassels of the corn were significantly (0.01 level) higher in the plots treated with 0.14 and 0.55 kg of 2,4-D per hectare than in the untreated plots. These numbers were for (i) 1420, (ii) 2449, (iii) 3116, and (iv) 2023. The percentages of corn plants attacked by the corn borer were 63 percent after (i), 83 after treatment (ii), 70 after treatment (iii), and 63 after treatment (iv). Differences between treatments of 0.14 and 0.55 kg of 2,4-D per hectare and the control were statistically significant (0.05 level).

In laboratory tests the single hybrid OH 51A × B8 corn was grown in a growth chamber at temperatures of 28° to 29°C. After 4 weeks (when the corn was 40 to 50 cm tall) 90 ml of 2,4-D solution was applied to the soil in each pot at concentrations of 0, 5, 20, 80, and 320 parts per million (ppm). The 20-ppm con-