N. Tanner, *J. Chem. Soc. Perkin Trans. 1*, **1972**, 1387 (1972).
12. L. J. Porter, *N.Z. J. Sci.* **17**, 213 (1974).
13. Finely ground *Pinus radiata* bark, previously extracted with hexane and then with toluene, was exhaustively extracted with ethyl acetate. Much of the higher molecular weight tannin material in the extract was removed by precipitation, first by concentration on a rotary evaporator at 30°C, then by fractional precipitation with hexane [T. A. Geissman and H. F. K. Dittmar, *Phytochemistry* **4**, 359 (1965)]. Catechin, procyanidins, and remaining higher molecular weight tannins were extracted into water and then filtered on a column of Sephadex G-25 (Medium) (88 by 5 cm), prepared and eluted with 50 percent aqueous acetone [L. J. Porter and R. D. Wilson, *J. Chromatogr.* **71**, 570 (1972)]. The early fractions (excluded from the gel) contained only the higher tannins and were freeze-dried for use in later growth studies. This preparation was shown by paper chromatography to be carbohydrate-free. The procyanidins and catechin were subsequently separated from one another by chromatography on a column of Sephadex LH-20 (74 by 2.6 cm), prepared and eluted with methanol. Traces of other phenolics in the preparations were removed by partition chromatography on columns of cellulose which were partially eluted with a mixture of butan-2-ol, acetic acid, and water (14:1:5) (*11*), then extruded and sliced [A. Thompson, in *Methods in Carbohydrate Chemistry*, R. L. Whistler and M. L. Wolfrom, Eds. (Academic Press, New York, 1962), vol. 1, p. 36]. After a final cleanup of each preparation by gel filtration on Sephadex G-25, they were then tested for homogeneity by two-dimensional paper chromatography (*11*) and freeze-dried. Yields were 0.26 g (catechin), 1.51 g (B-3), and 0.17 g (C-2) from 1 kg of bark.
14. E. C. Bate-Smith, *Phytochemistry* **12**, 907 (1973); E. Haslam, *Biochem. J.* **139**, 285 (1974).
15. Flask contents were filtered through Whatman paper No. 54. Filtrates were adjusted to 5.0 ml and frozen. Mycelia were washed five times with 5.0 ml of distilled water each time at 40°C, dried overnight at 110°C, and then analyzed for total cell nitrogen by a Kjeldahl procedure. Figures for mycelial dry weights were obtained from a calibration curve relating dry weight to total cell nitrogen prepared with the *Penicillium* grown on glucose in the salts medium, using the harvesting and washing procedure already described. Residual catechin or B-3 in culture filtrates and in uninoculated controls was assayed by gas-liquid chromatography of their trimethylsilyl (TMS) derivatives according to a method adapted from that of Eastmond [R. Eastmond, *J. Inst. Brew. (London)* **80**, 188 (1974)]. As the TMS derivative of the C-2 trimer was insufficiently volatile for such an assay, C-2 was estimated by semiquantitative paper chromatography of samples of the filtrates with a range of standards on the same paper. Two-dimensional paper chromatograms of all culture filtrates showed that interference in the assays by traces of other phenolic products was negligible.
16. Fifty to 60 percent of the tannins [M. J. Taras, A. E. Greenberg, R. D. Hoak, M. C. Rand, Eds., *Standard Methods for the Examination of Water and Waste Water* (American Public Health Association, Washington, D.C., 1971), p. 346] in uninoculated control flasks was precipitated from solution after 7 days of incubation and aeration.
17. I thank Cushla McMurtry for her technical assistance, and Dr. Lawrence J. Porter for his advice and for providing samples of procyanidins for reference purposes.

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Behavioral Fever in Newborn Rabbits

Abstract. *Rabbit pups, 12 to 72 hours old, did not develop a fever when injected intraperitoneally with a pyrogen and maintained at an ambient temperature of 32°C for 2 hours. When placed in a thermally graded alleyway, animals injected with pyrogen selected gradient positions that represented significantly higher temperatures than controls injected with saline (40.4° in contrast to 36.4°C). Allowing the pups to remain at their selected positions for 5 minutes caused a significant increase in the rectal temperatures of the pyrogen-injected pups but not that of the controls. Thus, newborn rabbits will develop a fever by behavioral means after a single injection of an exogenous pyrogen.*

Unlike adults, newborn mammals do not develop a fever upon initial exposure to a bacterial pyrogen (*1*). Neonates do not respond well to thermal stresses in general, and can maintain normal body temperatures only within a narrow range of environmental temperatures. In this respect, infant mammals are very much like reptiles, which have inadequate or nonexistent physiological thermoregulatory mechanisms. Reptiles, however, will maintain their body temperatures within narrow limits when given the opportunity to do so behaviorally (*2*). Many infant mammals also show thermal preferences from the day of birth and will maintain normal body temperatures by moving to the appropriate place in a thermal gradient (*3*). In response to a bacterial infection, iguanas will develop a fever by spending more time at higher environmental temperatures (*4*). Fish also show a behavioral fever when injected with a pyrogen (*5*). We report here that, in response to an initial challenge with a pyrogen, newborn rabbits, unable to develop a fever physiologically, will do so behaviorally by selecting higher environmental temperatures than nonchallenged controls do.

Eleven New Zealand white rabbit pups from two litters were used in these experiments. They were 12 to 72 hours old and weighed between 50 and 89 g. The pups were removed from their mothers and divided into three groups matched as closely as possible for body weight. One group was given an intraperitoneal injection of *Pseudomonas* polysaccharide (Piromen), 500 μg per kilogram of body weight, dissolved in sterile saline (250 μg/ml). The second group received the saline vehicle alone (2 ml/kg), and the third group was given no treatment. After injection, the pups were placed in individual containers in an incubator kept at 32°C. Two hours after injection, rectal temperatures were measured with a 36-gauge, copper-constantan thermocouple inserted 2.0 cm into the rectum. The pups were then placed, two at a time, in a temperature gradient apparatus similar to that used by Ogilvie and Stinson (*3*). This consisted of an alleyway whose bottom was a copper bar (183 by 15 by 0.64 cm) with aluminum sides (122 cm long, 15 cm high) and a hinged Plexiglas top. At each end of the alleyway, the bar extended 31 cm. Heating tape was wrapped around one end, and temperature was controlled with a Variac voltage transformer. The temperature gradient along the alleyway ranged from 22° to 55°C. Thermocouples were placed every 2.5 cm along the gradient, and 49 divisions were marked along the sides of the enclosure corresponding to the thermocouple placements. The pups were placed in the gradient with their noses touching a point corresponding to 30°C; half were positioned facing the hot end and half facing the cool end. The position of each animal in the gradient was recorded every minute, as were the temperatures under the thermocouples. An experiment, which generally took no more than 25 minutes, was terminated when a pup remained at the same place in the gradient for at least 5 minutes. When a pup was removed from the gradient, its rectal temperature was again recorded, and it was returned to its mother. No rabbit in any group was given more than a single injection of Piromen. The rabbits getting no injection on day 1 received Piromen on day 2 and saline on day 3; those receiving saline on day 1 got no injection on day 2 and Piromen on day 3.

To ensure that the dose of Piromen used was sufficient to give older rabbits a fever, the pups were divided into two groups, matched for body weight, when they were 14 days old. Five were injected with Piromen (500 μg/kg) and six with a similar volume of saline alone. All the pups were maintained individually in the incubator at 27°C, and rectal temperatures were taken before and 2 hours after injection.

All data are reported as the mean (± standard error of the mean) unless otherwise indicated. Student's *t*-tests were performed on the data, and the null hypothesis was rejected when $P \leq .05$.

The main results of the experiment are shown in Fig. 1a. Rabbit pups injected with saline selected a gradient temperature of 36.5° ± 0.47°C. Pups injected with Piromen selected a significantly higher gradient temperature,

40.4° ± 0.76°C ($P < .001$). No pup injected with saline selected a gradient temperature higher than 38.0°C, whereas nine of the 11 pups injected with Piromen selected temperatures higher than 39.9°C. Two of the 11 Piromen-injected animals did not respond to the pyrogen. The reasons for this are not obvious, since the body weights, initial rectal temperatures, and general mobility of all the pups were the same. Uninjected pups selected a slightly lower mean gradient temperature (35.4° ± 0.46°C) than did saline controls, but the difference was not significant.

The mean rectal temperatures of all three groups were within 0.5°C of each other (37.2° to 37.7°C) just before they were placed in the thermal gradient. Since this was 2 hours after treatment, it indicates that the group receiving Piromen was not able to develop a fever physiologically at 32°C within this time period. When the pups were removed from the alleyway, after 5 minutes at their selected positions, the mean rectal temperature of the Piromen-treated group was significantly higher than it had been prior to testing (38.6° ± 0.29°C compared to 37.7° ± 0.25°C; $P < .005$). There was no significant change in rectal temperature in either of the control groups.

The increase in body temperature was achieved solely by selecting higher gradient temperatures. Figure 1b shows the gradient temperature selected in relation to the pups' core temperatures just prior to testing. The saline-treated group selected a gradient temperature about 1°C below its starting core temperature. The Piromen-treated group selected a gradient temperature 2.8° ± 0.9°C above its body temperature before the test. This difference was significant ($P < .001$).

The pups were extremely mobile, even 12 hours after birth, and their behavior was highly consistent. When first placed in the alleyway at the 30°C position, they immediately moved toward the hotter end, regardless of whether they had been placed facing toward or away from that end. They often moved past the point corresponding to their final preferred position. Then they either turned immediately and began moving toward the cooler end, or rested at the high temperatures for up to 2 minutes before moving down to a position cooler than their final choice. This oscillatory behavior lasted for 10 to 20 minutes, after which the animals remained in their final selected positions for at least 5 minutes. There was no significant difference between the two groups either in the time taken to reach the final positions or in the time spent at temperatures warmer or cooler than the final selections. When the pups rested at nonpreferred temperatures, they made postural adjustments similar to those of adults. At high gradient temperatures they assumed a sprawled posture; at cooler temperatures they curled up into a ball-like posture identical to that reported for adult rabbits (*6*). At the final selected temperatures the pups usually lay on their sides, often in a semicurled position.

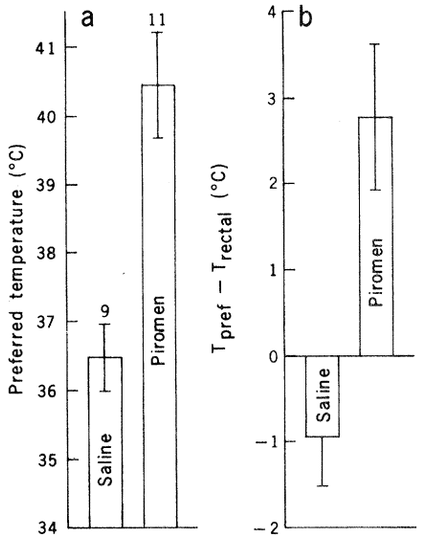

Fig. 1. (a) Preferred gradient temperature (°C) as a function of saline or pyrogen injection. Numbers over bars are number of pups in each group; bars are ± 1 S.E.M. (b) Preferred gradient temperature (T) minus pretest core temperature as a function of saline or pyrogen injection.

When they were 14 days old, five rabbits were injected with Piromen and six with the saline vehicle. All were kept in the incubator at 27°C. At the end of 2 hours, the rectal temperatures of the animals injected with saline rose from a mean of 38.1° ± 0.19°C to 39.1° ± 0.09°C, a rise of 1° ± 0.23°C. The rectal temperatures of the Piromen-injected animals rose from 37.9° ± 0.11°C to 39.9° ± 0.12°C, an increase of 2.0° ± 0.19°C. The difference between the initial and final temperatures of the animals injected with saline in contrast to that for animals injected with Piromen was significant ($P < .005$). Thus, the drug, dose, and route of administration used for neonates were adequate to generate a fever physiologically when the pyrogen was injected a second time when the rabbits were older.

This experiment demonstrates that exogenous pyrogen will produce a fever in naive newborn rabbits if the opportunity for thermoregulatory behavior is present. No fever develops if the pups must rely solely on internal thermoregulatory mechanisms. According to current concepts, fever develops when a bacterial pathogen, interacting with white blood cells, causes the release of leukocytic pyrogen. The leukocytic pyrogen travels through the bloodstream to the hypothalamus where it changes the activity of temperature-sensitive neurons in such a way that heat production is increased and heat loss is suppressed. This, in turn, raises body temperature (*7*). Until the present experiment, the absence of fever in newborn mammals could have been attributed to (i) the leukocytes' inability to manufacture endogenous pyrogen, (ii) the immaturity of the hypothalamic thermal control system, or (iii) the inadequacy of the effectors to increase heat production reflexively and so offset the heat lost because of lack of insulation (newborn rabbits have little fur) and an unfavorable surface-to-volume ratio. The present experiments suggest that the third possibility is most probable; the drive toward fever is present, but heat-producing and heat-conserving mechanisms are inadequate. Alternatively, early in development there may be separate neural mechanisms for behavioral and physiological thermoregulation. The behavioral system for producing a fever is clearly mature at birth, but an adequate system of internal reflexes does not appear to develop for some days.

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References and Notes

1. K. E. Cooper, Q. J. Pittman, W. L. Veale, in *Temperature Regulation and Drug Action*, P. Lomax, E. Schönbaum, J. Jacob, Eds. (Karger, Basel, 1975), p. 43; C. M. Blatteis, *J. Appl. Physiol.* **39**, 251 (1975).
2. C. B. DeWitt, *Physiol. Zool.* **40**, 49 (1967).
3. For pigs, see L. E. Mount, *Nature (London)* **199**, 1212 (1963); for mice, D. M. Ogilvie and R. H. Stinson, *Can. J. Zool.* **44**, 511 (1966); and for rabbits, E. Jeddi, *Int. J. Biometeorol.* **15**, 337 (1971).
4. L. K. Vaughn, H. A. Bernheim, M. J. Kluger, *Nature (London)* **252**, 473 (1974).
5. W. W. Reynolds, M. E. Casterlin, J. B. Covert, *ibid.* **259**, 41 (1976).
6. G. N. McEwen, Jr., and J. E. Heath, *J. Appl. Physiol.* **35**, 884 (1973).
7. R. J. Hellon, *Pharmacol. Rev.* **26**, 289 (1975).

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