after even very short digestion times when isolated chromatin is used. Also, the time course of digestion is much more rapid in isolated chromatin. Both of these observations could be explained by the increase in accessibility of the nuclear chromatin in solution. However, it is significant that much of the same repeating structure still exists for the in vitro chromatin.

The width of the DNA monomer band in higher eukaryotes does not preclude microheterogeneity of these particles. The sharper yeast monomer bands, especially for the in vitro chromatin digests, suggest a much less polydisperse subunit.

As in higher eukaryotes, these size classes probably arise as a result of protection of sections of DNA by bound chromosomal proteins, rendering these sections inaccessible to nucleases. Evidence indicates that in mammalian systems these protected sections occur as repeating, globular particles in chromatin (*12*), and models for the structure of such particles have been proposed (*13, 14*). The presence of specific DNA size classes, and presumably a similar repeating subunit structure, in one of the most primitive eukaryotes indicates that this mode of organization must be a widespread characteristic of the structure of chromatin. Of course, the fine structure of such a subunit in yeast may very well differ from the subunit of higher eukaryotes, especially since histones F1 and probably F3 are lacking (*6*). If this is true, F3 cannot fulfill, in the yeast chromosomal subunit, the central role postulated for it by one model [see (*13*)] of the chromosomal subunit in higher eukaryotes. The presence of a periodic mode of chromatin organization in yeast, in which a relatively large proportion of the DNA corresponds to genetically expressed information, suggests that this mode of organization may involve structural genes as well as (or perhaps instead of) other types of nuclear DNA.

DENNIS LOHR
K. E. VAN HOLDE
Department of Biochemistry and Biophysics, Oregon State University, Corvallis 97331

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10. On gels we used, size has been tentatively estimated relative to fragments of PM2 DNA produced by Hae III restriction endonuclease. These fragments were generously supplied by R. Kovacic. Figures 1 and 2 show that the basic band size centers around 135 base pairs. The (broader) calf thymus monomer band centers around 150 base pairs on similar gels (*3*). A monomer band size of 205 base pairs has been reported from rat liver (*2*). Exact comparison is difficult at this time because different standards have been used for size estimation. Furthermore, differences in size due to the different systems studied cannot be excluded.
11. Some workers have plotted log of band number versus the square root of gel mobility to show such molecular weight relations [R. Williamson, *J. Mol. Biol.* **51**, 157 (1970); D. R. Hewish and L. A. Burgoyne (*1*)], while log plots of molecular weight versus the first power of mobility are widely used in restriction endonuclease studies [see E. Huang, J. Newbold, J. S. Pagano, *J. Virol.* **11**, 508 (1973)]. Our results fit better the plot of log band number versus mobility; plots against the square root of mobility also give straight lines but the fit is not as good as for the first power plots. Since all such plots are empirical and lack firm theoretical justification, we have chosen to use the first power of mobility plots.
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Fever and Survival

Abstract. *The significance of fever in response to a bacterial infection has been investigated using the lizard* Dipsosaurus dorsalis *as an animal model. These lizards develop a fever of about 2°C after injection with the bacterium* Aeromonas hydrophila. *To determine whether this elevation in body temperature increases the resistance of the host to this infection, as measured by survival, lizards were infected with the live bacteria and placed in a neutral (38°C), low (34° or 36°C), or high (40° or 42°C) ambient temperature. An elevation in temperature following experimental bacterial infection results in a significant increase in host survival.*

The significance of fever in disease is an enigma (*1–3*). Most mammals develop fever after becoming infected with bacteria; but it is unclear whether the fever is harmful or beneficial to the host. Bennett and Nicastri (*1*) reviewed arguments for and against the common notion that fever is advantageous to the host in combating infection. Studies with specific diseases (syphilis and gonorrhea) involved extraordinarily high and therefore unnatural elevations in body temperature, which do kill the parasites before the host and therefore are beneficial to the host ("fever therapy") (*1*). Other studies (*4*) involved rendering an animal hyperthermic or hypothermic and then measuring its resistance to infection. These approaches are not directly relevant to the question whether fever benefits the host because in fever therapy the temperature alterations, which are artificially induced, exceed the normal range of the febrile response, and in the experiments involving resistance to infection the temperature alterations precede the infection. A more direct test would involve determinations of LD_{50}'s (LD_{50} is the lethal dose to 50 percent of a population) in two groups of animals injected with suitable bacteria. In one group, the febrile response would be allowed to develop, and in the other group the fever would be suppressed. Fever could be suppressed by administering an antipyretic drug such as aspirin, by artificially elevating the hypothalamic temperature, by placing the animals in an ice bath, or by maintaining them in a

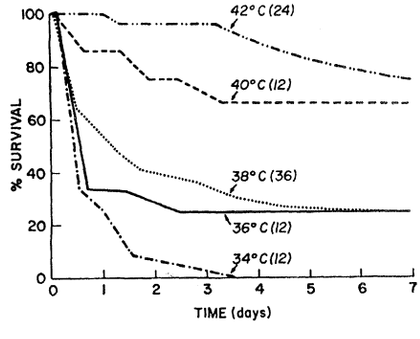

Fig. 1. Percentage survival of *D. dorsalis* injected with *A. hydrophila* and maintained at temperatures of 34° to 42°C. The number of lizards in each group is given in parentheses.

low ambient temperature. However, all of these methods of suppressing fever tend to produce complicating side effects.

Reptiles, which regulate their temperature by behavioral means, might serve as a suitable animal model to investigate the adaptive value of fever. For example, the lizard *Dipsosaurus dorsalis*, when placed in a chamber which has a temperature range, will select a "preferred" ambient temperature so that it has a mean body temperature of 38.5° to 39.0°C (*5, 6*). If *Dipsosaurus* is placed in a constant temperature chamber, where there is no opportunity to select its preferred temperature, its body temperature equilibrates with the temperature of that chamber. This permits full control of the animal's temperature under a wide variety of experimental situations. Furthermore, the febrile response, which has been found to involve physiological and behavioral modifications in mammals (*7*), has recently been shown to occur in lizards by behavioral modifications alone (*8, 9*). *Dipsosaurus* develops a 2°C fever through behavioral means following injection of a killed bacterial pathogen, *Aeromonas hydrophila* (*8, 9*). Lizards have two distinct advantages over mammals in a study involving the adaptive significance of fever. First, a change in body temperature of a few degrees does not have adverse effects in reptiles, as it does in mammals. Lizards normally regulate their temperature over a range of more than 8°C, whereas in most mammals this range is about 1°C. Second, a lizard's body temperature can be kept constant after pyrogen administration by simply controlling the ambient temperature, while in mammals fairly drastic procedures must be employed to prevent fever. Thus, lizards and other behavioral thermoregulators have advantages as animal models for evaluating the role of fever in disease.

In this report we describe a study of the role of fever in which we used *Dipsosaurus dorsalis* as our experimental animal. We found that in lizards inoculated with live bacteria, an elevation in body temperature resulted in increased survival.

A total of 141 lizards was used. The inoculum of live *Aeromonas hydrophila*, a gram-negative bacterium pathogenic for reptiles (*10*), was grown at 37°C for 14 to 18 hours on sheep blood agar plates. The bacteria then were suspended in sterile pyrogen-free physiological saline and washed twice by centrifugation, decantation, and resuspension. They were then diluted with sterile pyrogen-free saline to yield a concentration of approximately 1×10^{10} organisms per milliliter, as determined by comparing both visual and photometric turbidity with McFarland barium sulfate standards. Pour plates revealed that the concentration of live organisms was approximately 1 log unit less than indicated by turbidity. Preliminary studies revealed that 0.5 ml of this bacterial suspension injected in the middorsal lymph sac produced significant mortality in lizards maintained at 38°C for 1 week. At necropsy, microbiological cultures of heart blood and viscera of these animals contained pure colonies of *A. hydrophila*.

Each lizard was inoculated with 0.5 ml of the bacterial suspension (1×10^9 live bacteria per milliliter) and placed in a glass culture dish (250 mm in diameter and 80 mm high). Groups of 12 lizards were housed in incubators at 34°, 36°, and 40°C; 24 were placed at 42°C; and 36 were placed at 38°C. Control animals were inoculated with 0.5 ml of sterile pyrogen-free saline and maintained at 34°C ($N = 12$), 38°C ($N = 12$), and 42°C ($N = 21$).

The lizards were monitored for mortality every few hours throughout a 7-day period, and among those that died necropsies were performed on representative animals. Tissue samples of the heart, brain, liver, lung, kidney, gastrointestinal tract, urogenital tract, skeletal muscle, skin, and bone were fixed in 10 percent buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. The sections were then examined by light microscopy. At the end of the 7-day period, representative survivors were killed and necropsied and tissues were prepared and examined histologically.

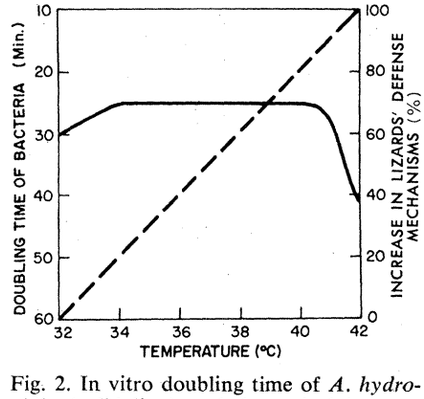

Fig. 2. In vitro doubling time of *A. hydrophila* (solid line) and theoretical increase in the lizards' defense mechanisms (dashed line) plotted against temperature. At 34°C, the growth of the bacteria far outweighs the lizards' ability to destroy these organisms, and all animals die. At 36° to 38°C, this difference decreases and the survival increases to 25 percent. Between 38° and 40°C, the lizards' defense mechanisms have improved so that a majority of the lizards survive the infection, even though the in vitro growth of *A. hydrophila* is unchanged from that in the range 34° to 38°C. At 42°C, the enhanced defense mechanisms together with a diminished bacterial growth rate result in essentially no deaths attributable to the bacterial infection.

Growth rates of *A. hydrophila* at different temperatures were determined by inoculating 14- to 16-hour cultures into shaking flasks of brain-heart infusion broth at the five experimental temperatures as well as at 44°C. Increase in absorbance (total bacterial mass) was measured periodically in samples of the growing cultures. The doubling time for these organisms was essentially the same (22 to 28 minutes) at 34°, 36°, 38°, and 40°C; increased to 42 minutes at 42°C; and increased to more than 180 minutes at 44°C.

The results of the 7-day experiments on the infected lizards are shown in Fig. 1. The relation between the lizards' temperatures and percentage survival following bacterial infection was highly significant (χ^2 test, $P < .005$). Within 24 hours, approximately 50 percent of the lizards maintained at 38°C were dead. However, lizards maintained at 40° and 42°C had only 14 and 0 percent mortality, respectively. Conversely, lizards maintained at 36° and 34°C experienced increased mortalities of 66 and 75 percent, respectively. By 3.5 days all the lizards at 34°C were dead. After 7 days the percentage mortalities were: 42°C, 25; 40°C, 33; 38° and 36°C, 75; and 34°C, 100. By contrast, lizards injected with saline and maintained at 34°, 38°, and 42°C for 7 days experienced 0, 0, and 34 percent mortality, respectively.

Tissues of six lizards that died within 3 days after injection of *Aeromonas* showed no significant microscopic lesions, and death was attributed to acute septicemia. Of 24 infected lizards that survived 3 to 7 days, statistically significant differences between groups were seen in the incidence of coelomitis (higher in the groups at 34° to 38°C) and testicular degeneration (higher in the groups at 40° and 42°C). A greater percentage of lizards held at 34° to 38°C had lesions consisting of purulent myositis in skeletal muscle at the site of injection of *Aeromonas* (7/15 or

46.7 percent compared to 1/9 or 11.1 percent in lizards held at 40° and 42°C). There were no significant differences between groups in the incidence of skin lesions at the injection site (necrosis, ulcers, or gangrene).

The increased survival of infected animals with elevated body temperature supports the hypothesis that fever following a bacterial infection (*8, 9*) is beneficial to the host. Inasmuch as the in vitro bacterial growth rate was stable between 34° and 40°C, the increased survival of the lizards at 40°C could be attributed to an enhancement of the host's defense mechanisms at the elevated temperature. The specific aspects of the defense mechanisms that might improve with increasing temperature remain unknown. It is possible that several components of the defense mechanisms, including phagocytic index, phagocyte bactericidal activity, leukocyte mobilization, and humoral mediators of inflammation, are temperature-dependent. It is also possible that the toxigenicity of the bacteria decreased as the temperature increased. At 42°C, the decreased bacterial growth rate probably also contributed to the increased survival. Conversely, the reduction in temperature below normal levels (such as to 34°C) following bacterial infection led to increased mortality, possibly due to impaired host defense. Our interpretation of these data is shown in Fig. 2.

At the highest temperature tested, the pattern of deaths was similar for the controls and the infected lizards. Whereas most deaths occurred within 3.5 days in infected lizards maintained at 34° to 40°C, essentially all deaths at 42°C occurred after 3.5 days. Apparently, maintenance at 42°C for a period exceeding 3.5 days is harmful in itself. This suggests that the deaths at 42°C were not due to the bacterial infection but to some undetermined adverse effect of long-term elevation in temperature.

We believe these data may have relevance for mammals. Assuming a common phylogenetic origin of fever in present-day mammals and reptiles, there is reason to expect that the function of fever is similar in these two vertebrate classes. That is, if fever evolved in reptiles as a mechanism to decrease mortality and morbidity following infection, its function should be similar in mammals. If fever in response to infection is beneficial in mammals, then the widespread use of antipyretics to lower the temperature of people with moderate fevers should be reevaluated (*11*).

MATTHEW J. KLUGER
Department of Physiology, University of Michigan Medical School, Ann Arbor 48104

DANIEL H. RINGLER
MIRIAM R. ANVER
Unit for Laboratory Animal Medicine, University of Michigan Medical School

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11. Clinical observations suggest that an elevation in temperature, following bacterial infection, is beneficial to humans. In certain clinical situations the use of antipyretics is contraindicated. For example, bacterial infections often develop in patients with third-degree burns. As destruction of epithelial layers leads to an increased evaporative heat loss, these patients often encounter thermoregulatory difficulties. The prognosis for survival is considered better in patients developing a fever of 1° to 2°C than in patients who remain at the normal or afebrile body temperature (I. Feller, personal communication).
12. We thank L. D'Alecy, A. Vander, and B. Cohen for their critical evaluation of this research and S. Cooper for his assistance in determining the growth rates of *Aeromonas*. We also acknowledge the technical assistance of J. Park and A. Sofen. Supported by NSF grant GB 42749X0 and NIH grant RR-00200.

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HeLa Cells and RT4 Cells

In a note added in proof to their report on cellular contamination in tissue culture, Nelson-Rees *et al.* (*1*) reported that cells of culture line RT4 derived from a human bladder tumor (*2*) had features which suggested that the cells were HeLa cells. We have reexamined the RT4 cell stock maintained in our laboratories. These cells have a modal chromosome number of 47 and glucose-6-phosphate dehydrogenase, type B; they also differ from HeLa cells at three other loci tested, namely, phosphoglucomutase 1, phosphoglucomutase 3, and esterase D. The parent stock of RT4 cells therefore is not HeLa cells (*3*). It should be pointed out that the cells examined by Nelson-Rees *et al.* had been maintained in other laboratories for some years and were not provided directly from our parent stock.

L. M. FRANKS
Department of Cellular Pathology, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England

CAROLYN RIGBY
Department of Pathology, St. Paul's Hospital, London WC2

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3. We thank the staff of the MRC Human Biochemical Genetics Unit, Galton Laboratory, University College, London (Dr. S. Povey), and Dr. C. W. Parr, London Hospital Medical School, for the iscenzyme studies.

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The comment by Franks and Rigby emphasizes two points: (i) It is relatively easy to check specificity of cells and (ii) distributing cells without characterizing them whether or not the distributor is the originator is as wrong as finding out that cells are contaminated and not announcing where they came from.

In our report in *Science*, courtesy led us to keep the sources of the cell cultures anonymous, although we had originally cited them when communicating with all parties concerned prior to submitting the manuscript.

WALTER A. NELSON-REES
Cell Culture Laboratory, University of California, School of Public Health, and *Naval Biomedical Research Laboratory, Oakland 94625*

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