Life at High Temperatures

Evolutionary, ecological, and biochemical significance of organisms living in hot springs is discussed.

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Temperature is one of the most important environmental factors controlling the activities and evolution of organisms, and is one of the easiest variables to measure. Not all temperatures are equally suitable for the growth and reproduction of living organisms, and it is, therefore, apt to consider which thermal environments are most fit (*1*) for living organisms. For such a study, high-temperature environments are of especial interest, in that they reveal the extremes to which evolution has been pushed. The high-temperature environments most useful for study are those associated with volcanic activity, such as hot springs, since these natural habitats have probably existed throughout most of the time in which organisms have been evolving on earth.

A recent survey of the thermal waters of the world (*2*) provides a broad view of their distribution. The antiquity of many individual springs is noteworthy. Historical records of springs in the Mediterranean go back to the ancient Greeks and Romans (*3, 4*). Chemical studies on mineral springs were initiated by Robert Boyle in the 17th century, and were greatly systematized in the 19th century by Bunsen (*5*), who was apparently the first to see the value of chemical studies in interpreting the origin of hot springs. The early geochemical work is reviewed by Allen and Day (*6*). Chemical analyses have also been made by balneologists seeking an explanation of the reputed curative properties of certain springs (*7*). Unfortunately for the biologist, many chemical elements of biological significance are not assayed by either the geochemist or balneologist, but the results of the analyses do show that there are many chemical types of hot springs. The *p*H of hot springs varies and values as low as 1.0 and greater than 9.0 have been recorded (*8*). Many, but by no means all, hot springs have significant amounts of hydrogen sulfide. The concentration of such interesting elements as fluoride, arsenic, rare earths, and gold varies very much from spring to spring. Many springs are highly radioactive (*9*), whereas others have no more radioactivity than normal ground waters. Some springs precipitate silica, others deposit travertine ($CaCO_3$), and still others form elemental sulfur. When one considers chemical, hydrologic, thermal, and geographical variation (*10*), it is clear that every hot spring can be considered as an individual, differing in minor or major ways from other springs. However, many springs are more similar than different. For instance, in the geyser basins of Yellowstone National Park, virtually all springs contain mildly alkaline waters which deposit silica (*6*), although even these springs show differences in that they contain varying and significant amounts of dissolved gases, including the biologically important gas methane. Some springs have been remarkably constant in thermal, chemical, and hydrologic properties for many years, although minor variations do occur (*11*). This constancy or steady-state condition (*12*) attracts the ecologist, because it eliminates many of the complications which interfere with a sophisticated analysis of ecological relationships. Further, many springs form relatively well-defined outflow channels, and, as water cools along these channels, relatively stable thermal gradients are created. In the thermal gradient of a single spring, under appropriate conditions, the only significant variable may be temperature (*13*). We thus have excellent naturally occurring experimental conditions, and we can ask questions about the ecology and evolutionary relation of organisms at different temperatures along these thermal gradients (*14*).

Most of the surface of the earth has a moderate temperature, with an average of 12°C (*15*). Biologists have dealt, for the most part, with organisms living at these temperatures, and most of our ideas of the temperature relations of organisms have been influenced by this fact (*16*). Especially ideas concerning the thermal instability of enzymes and other proteins probably arise from the fact that those proteins studied have come from organisms that live always at moderately low temperatures.

Upper Temperature of Life

In antiquity, the presence of organisms in hot springs was noted. Pliny the Elder, in his Natural History, noted: "Green plants grow in the hot springs of Padua, frogs in those of Pisa, fishes at Vetulonia in Etruria near the sea" (*17*). The hot springs at Padua (Abano) still flow, and they are colonized by blue-green algae, perhaps the green plants of Pliny. The algae of hot springs were described by many early workers (*18*), but it was Ferdinand Cohn (*19*) who first realized the general biological significance of organisms living in hot springs: "Even simple visual observations of the different colors show that different species exist at different temperatures in the water. Such observations are not merely of passing interest, since even if most aquatic plants and animals cannot live at temperatures above 37°C . . . it is important to know the highest temperature at which organic life, *no matter how organized,* can exist." He did not find any algae growing in Karlsbad waters at temperatures above 55°C, a condition which generally agrees with Agardh's earlier observations and Löwenstein's later ones (*20*).

Hoppe-Seyler (*21*) extended Cohn's work and pointed out two basic technical problems: (i) The necessity of showing that the organisms are indeed growing at the temperature in question; and (ii) temperature readings must be taken precisely where the or-

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ganism is found, since the temperature even a few centimeters away from the organism may be quite different from that of the organism. From his observations on the hot springs of Padua (Abano), Sicily, and Ischia (Bay of Naples), he concluded that the upper temperature for algal growth was just above 60°C. He saw that his observations might have significance for an understanding of the origin of life and speculated that when the earth was cooling, chlorophyll-containing and hence CO_2-fixing and O_2-producing organisms could already live when the temperature was still over 60°C.

Before further discussion, it is necessary to examine what is meant by the question, what is the upper temperature of life? Temperature is only one of many variables influencing the growth of living organisms. It seems reasonable that environmental factors such as *p*H, nutrient quality or quantity, hydrostatic pressure, salinity, and light intensity (for photosynthetic organisms) will probably influence temperature responses, including the upper temperature at which an organism can grow. Heterotrophic bacteria cannot grow in hot-spring water in the absence of organic matter. We can thus imagine that the highest temperature at which some organism is now living somewhere on earth is not the upper temperature for life, but only the upper temperature at which all conditions for life are possible.

Probably the most careful early observations were those made by Setchell (*22*); these were apparently never published in detail. He made extensive observations in Yellowstone Park and reported that the upper temperature where algae were visible was 75° to 77°C, and that at which bacteria were found was 89°C. These upper limits occurred in siliceous waters having a slightly alkaline *p*H. In travertine-depositing springs, the upper temperature for algae was lower by about 10°C. In 1963, Kempner (*23*) reexamined this question, and cast doubt on Setchell's observations, suggesting that 73°C was the highest temperature at which organisms were found growing, but my own observations in Yellowstone confirm Setchell's conclusions. In the summer of 1966, Castenholz and I, together and separately, made a series of observations in Yellowstone. I have carried these considerably further in the summer of 1967. In the effluent channels of hot springs where the flow and temperature are usually constant, it is quite easy to determine the upper temperature for algal growth, the algal mats forming characteristic V-shaped patterns, which are due to the lower temperatures at the edge than those in the center of the channel (see Fig. 1). We found visible algal growth (of the unicellular blue-green *Synechococcus*) at temperatures up to 73° to 75°C, but not at higher temperatures (*24*) (Fig. 2). That these algae are growing and not merely existing can be easily shown by darkening the channel; within 5 to 7 days the algal cells have completely disappeared (*25*). This is due to the fact that a steady-state exists between growth and wash-out of the algal cells. In the absence of light, growth no longer can occur (thus showing that in nature these algae are obligately phototrophic), and the existing algae are quickly washed away (*26*).

Bacteria are present in some, but by no means all, of the hot springs at

Fig. 1. Lower Geyser Basin, Yellowstone National Park. In the foreground is a small spring showing in its effluent the characteristic V-shaped pattern of the algal mat. The center of the V is devoid of algae. The temperature at which algae are first seen is about 73°C, either at the point or the arms of the V. In the background, Great Fountain Geyser is erupting. [Photo by M. L. Brock]

Table 1. Approximate upper thermal limits for different groups of organisms.

Organism	Upper limit (°C)
Animals, including protozoa	45–51
Eucaryotic microorganisms (certain fungi and the alga *Cyanidium caldarium*)	56–60
Photosynthetic procaryotes (blue-green algae)	73–75
Nonphotosynthetic procaryotes (bacteria)	>90

temperatures much above 75°C. In the effluents of certain springs in the White Creek area I have found pinkish, yellowish, or whitish masses of filamentous bacteria at temperatures up to 88°C. These bacteria are present in such large amounts that they can be readily seen with the naked eye, and under the microscope are revealed as dense tangles of long filaments (Fig. 3). Spectrophotometry of acetone extracts of these organisms revealed no chlorophyll. In other springs, with temperatures of over 90°C, filamentous and rod-shaped bacteria are visible only microscopically. These organisms are probably similar to those described by Setchell as "filamentous Schizomycetes" although they are not sulfur bacteria, as he erroneously concluded, and are probably members of the Flexibacteria group (*27, 28*). Bacteria at temperatures "near 90°C" were also seen by Van Niel (*6*). Water boils at about 92°C at Yellowstone. That these bacteria are growing at these temperatures is shown by the fact that if an artificial substrate (a glass slide or piece of cotton string) is placed in the pool or effluent channel, it quickly becomes covered with bacterial cells (see Figs. 4–6). We have made a brief survey for bacterial growth in superheated pools of Yellowstone (at temperatures of 93.5° to 95.5°C) by immersing glass slides several feet down in these pools and retrieving them after a week to 10 days. In *every* pool, bacteria were present on the slides, and in over half the pools the bacteria so densely covered the slides as to form a film visible to the naked eye. In one pool, where the pink bacteria are present, string immersed in the pool (at temperatures which never go below 91°C) became covered with macroscopically visible pinkish masses.

In one spring (*Perpetual Spouter*, Norris Geyser Basin) in which bacterial development is very rapid on slides, we have examined the water itself for the presence of bacteria, by staining with acridine orange, filtering through membrane filters, and examining the filters with incident-light fluorescence microscopy. No bacteria were seen in a 100-milliliter sample. It is thus unlikely that the bacteria have merely become attached passively to the slides from the water. It is also unlikely that these organisms enter the pools from the air, as the organisms in different pools differ morphologically.

We thus see that bacteria are able to grow in Yellowstone at any temperature at which there is liquid water, even in pools which are above the boiling point. It is thus impossible to conclude that there is any "upper temperature of life."

Thermal Limits for Different Taxonomic Groups

We have already noted that nonphotosynthetic organisms grow at temperatures above those of photosynthetic ones. There are two ways in which we can obtain information on the upper temperature limits of different taxonomic groups. One is by looking at various thermal environments throughout the world, and the other is by looking at the species distribution along the thermal gradient in a single spring. In the latter case, we are dealing with organisms all living in water with the same chemical properties.

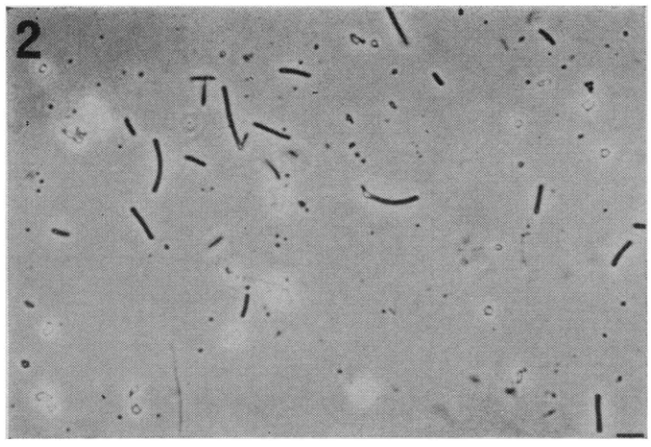
If we analyze the observations which have been made in all kinds of thermal environments (see *14, 18, 29* for references to the early literature) we can tentatively construct the scheme shown in Table 1.

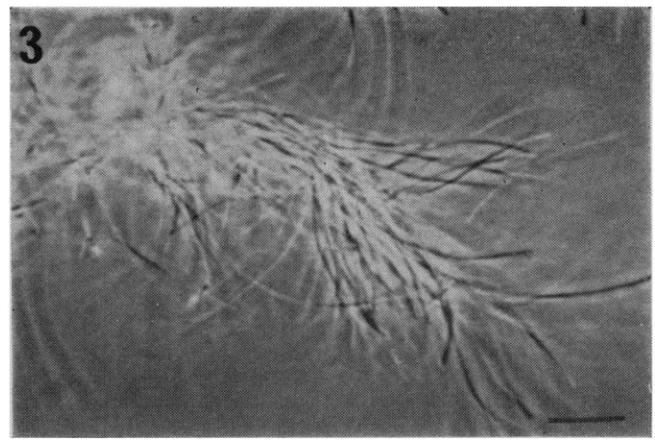
In evaluating these results, the problem of habitat suitability (other than temperature) and competition with other organisms merits attention. Thus, it is surprising that eucaryotic algae are not common at temperatures above 40°C, whereas eucaryotic fungi are found up to 60°C (*14*). This difference may merely be due to the fact that the niche into which the eucaryotic algae could fit is already occupied by blue-green algae. This idea is strengthened by the observation that the eucaryotic alga *Cyanidium caldarium* does live at temperatures near 60°C (*30*), but is restricted to very acid hot springs (*p*H less than 4) where blue-green algae do not grow. Even among the blue-green algae, competition can be seen. For instance, the cosmopolitan thermophile *Mastigocladus laminosus* lives at higher temperatures in Iceland than in many Yellowstone hot springs probably because it does not meet competition in Iceland from *Synechococcus*. In Yellowstone, many of the high-temperature environments in which *Mastigocladus* could grow are already well colonized with *Synechococcus*, and only where water flow is very rapid, and the unicellular *Synechococcus* cannot physically maintain itself, is *Mastigocladus* present in abundance at the higher temperatures (*31*).

Temperature Optima and Environmental Optima

It does not necessarily follow that these organisms actually prefer high temperatures as the name "thermophile" implies, since they may merely be lower temperature forms which have extended their ranges into thermal habitats where competition with less-resistant species is lacking (*32*). However, there has been little work attempting to relate temperature optima of thermophilic forms to environmental temperature. Most physiological work on thermophilic microorganisms has been done with bacteria isolated from soil, compost, or other habitats whose temperatures are variable or ill-defined. Hot springs provide ideal habitats for detailed studies because their temperatures are relatively constant and because the high heat capacity of water insures that the temperature of submerged organisms is the same as that of the water in contact with them.

Blue-green algae provide the best material, as their development in Nature is not as subject to the vagaries of water composition as are the bacteria, and because one of their most fundamental physiological processes, photosynthesis, can be measured easily with isotopes. Quantitative studies of the algal mats along thermal gradients in hot springs have shown a definite correlation between the temperature and the algal biomass (*33*). In the Yellowstone hot springs, maximum algal biomass was found at about 55°C, and





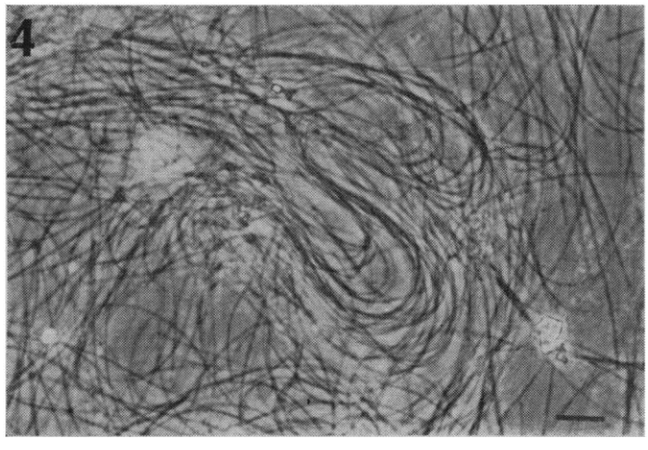
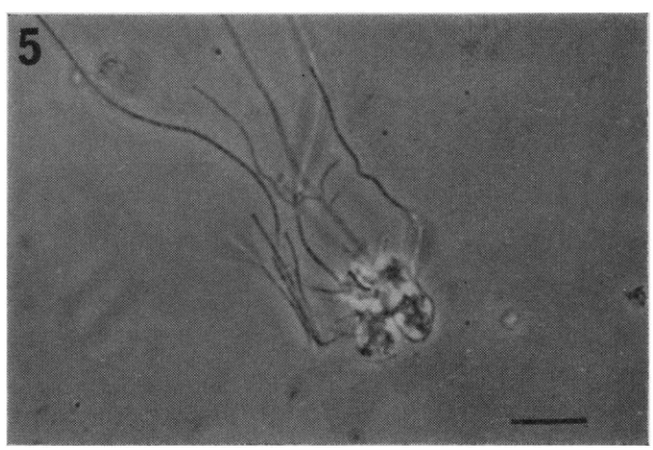


Fig. 2. Phase photomicrograph of microorganisms growing at 70° to 71°C in the outflow channel of Mushroom Spring, Yellowstone Park. The large cells are blue-green algae of the genus *Synechococcus*. There are also several filamentous bacteria visible, plus inorganic precipitate. The marker in the lower right-hand corner is 10 μ.

Fig. 3. Phase photomicrograph of filamentous bacteria taken from a large tuft in rapidly flowing water at 85° to 88°C in pool A, a large spring in the White Creek basin of Yellowstone Park. The marker is 10 μ.

Fig. 4. Phase photomicrograph of filamentous bacteria which grew on a glass slide immersed in Perpetual Spouter at about 90°C for 2 weeks. This field is representative of most areas of the slide. That these bacteria have grown on the slide is indicated by the manner in which the filaments are disposed on the slide. Detectable bacterial growth was seen on slides immersed only for a few hours. The marker is 10 μ.

it falls off sharply as the temperature increases above 55°C (*34*). At temperatures close to the upper temperature for algal growth, the biomass is exceedingly sparse. It was thus of interest to know if the algae living at these high temperatures were optimally adapted to those temperatures. The effect of temperature on the rate of photosynthesis was measured with $C^{14}O_2$ for algae living at different temperatures along the thermal gradient of a single spring (*35*). Typical temperature-rate curves, such as seen with individual species, were obtained, with well-defined optima. The optima were determined for a series of stations along the thermal gradient and then compared with the average environmental temperatures of these stations. In each case, the optimum temperature was quite close to the environmental temperature. This was true even for algae from 72°C, which are living at a temperature close to the upper limit for photosynthetic organisms. Thus, even though this temperature is



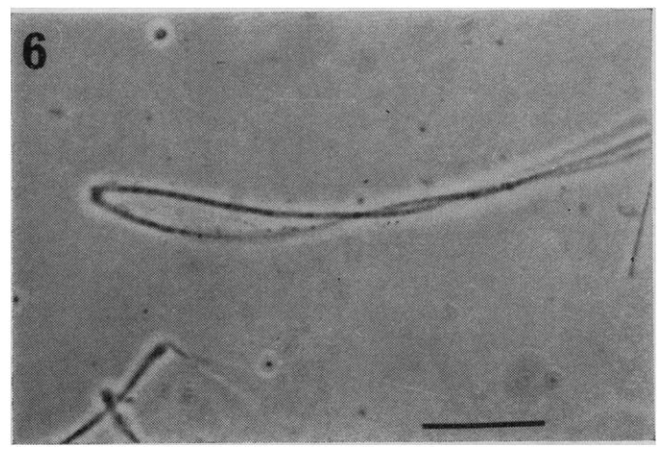


Fig. 5. Phase photomicrograph of bacteria which grew on a glass slide immersed in pool A (see Fig. 3) for 48 hours at 91.5°C. The marker is 10 μ. Fig. 6. Phase photomicrograph of a bacterial filament which grew on a glass slide immersed in pool A (see Fig. 3) for 48 hours at 91.5°C. The twisted filament is reminiscent of forms seen in some low-temperature Flexibacteria (*27*). The marker is 10 μ.

Table 2. Generation times of bacteria at their optimal growth temperatures.

Organism	Optimum temperature (°C)	Generation time (min)	Ref.
Vibrio marinus	15	81	(*54*)
Pseudomonas fluorescens	25	52	(*55*)
Bacillus megaterium	40	22	(*56*)
B. subtilis	40	26	(*56*)
Escherichia coli	40	21	(*44*)
Bacillus sp.	55	16	(*57*)
B. thermophilus	55	16	(*58*)
B. stearothermophilus	60 to 65	11	(*59*)
B. megaterium	70	13	(*56*)
B. coagulans	70	14	(*56*)
B. circulans	70	14	(*56*)

so extreme that algal development is quite sparse, the algae photosynthesize better there than they do at lower temperatures. This remarkable and fundamental observation suggests that the reason photosynthetic algae are not found on earth at temperatures higher than 73° to 75°C is because of some inherent limitation in the organization of their cellular material, a limitation impossible for them to overcome by further evolutionary changes.

Similar temperature-transfer experiments have been done with the bacteria living in association with the algae in a thermal gradient from 30° to 75°C, with analogous results. Studies have not yet been done with the bacteria living at temperatures over 90°C, so that it cannot be stated that these extreme thermophiles are optimally adapted to the temperatures at which they are growing.

Molecular Mechanism of Thermophily

Thus there are microorganisms that not only survive, but actually prefer, high temperatures. How can they live at these temperatures when proteins denature at much lower temperatures? However, concepts of protein denaturation have been developed through studies on proteins derived from mesophiles. Thermophiles have enzymes which, in general, are much more resistant to conditions which cause denaturation (*36–39*), just as the enzymes of mesophiles are much more stable than those of psychrophiles (*40*). Organisms seem to have enzymes which are stable throughout the temperature range in which they grow, but not at temperatures too much higher. Indeed, we might well turn the original question around and ask why psychrophiles and mesophiles have heat-labile proteins. There is in fact no reason to expect that a protein with any particular function could not exist at any high temperature at which its covalent bonds are stable. It is possible that induced fit and allosteric interactions between proteins and small molecules require a certain flexibility of structure which is incompatible with a highly cross-linked, rigid, and hence heat-stable protein. Thermophiles may thus have sacrificed efficiency and control of enzyme function in order to grow at high temperatures. There is some evidence for this idea. Thompson *et al.* (*41*) have observed that when the heat-stable aldolase of *Bacillus stearothermophilus* was treated with sulfhydryl compounds, the enzyme became considerably more heat sensitive, but at the same time the enzyme became more active at 30°C, although there was little change in the activity at 65°C. A most interesting area for investigation would be the study of the kinetics of a single enzyme common to a variety of organisms with different thermal optima. Finally, there is no reason that completely unfolded (and hence denatured) proteins cannot function as enzymes, and the alpha-amylase of *B. stearothermophilus* seems to be such an enzyme (*39*).

The stability of the protein-synthesizing machinery is likely to be of more importance than the stability of individual proteins, since there is nothing to replace the protein-synthesizing machinery if it is destroyed. A cell is not a phoenix, able to arise out of its own ashes. There is good evidence (*37, 42*) that the ribosomes and the protein-synthesizing machinery (as measured in vitro with systems of artificial messengers) of a thermophile are more stable than those of a mesophile. The heat stability of soluble RNA (sRNA) and DNA would never seem to be a problem, since even sRNA and DNA of mesophiles are quite stable in the kind of ionic environment found in the cell. Finally, there is no evidence that organisms are killed by heat because of the inactivation of proteins or other macromolecules. An analysis of thermal-death curves of various organisms shows that this is a first-order process. Thermal killing due to the inactivation of heat-sensitive enzymes or heat-sensitive ribosomes, of which there are many copies in the cell, should not result in simple first-order kinetics (*38*). However, first-order kinetics are compatible with an effect of heat on some large structure, such as the cell membrane, since a single hole in the membrane could result in leakage of cell constituents and subsequent death. In psychrophilic bacteria, thermal death (at temperatures around 25° to 30°C) seems to be due to damage to the cell membrane and subsequent lysis (*40*).

Hence the molecular mechanism of thermophily is possibly related to the stability of the larger membrane structures that are held together by the weak bonds that are likely to be broken by high temperature. The inability of eucaryotes to grow at temperatures as high as procaryotes may be due to the more complicated membrane systems of eucaryotes in their membrane-bound organelles: nuclei, mitochondria, chloroplasts. Extending this further, the reason why photosynthetic procaryotes cannot grow at temperatures as high as nonphotosynthetic ones may be because of the greater complexity of the photosynthetic membrane system. Even among the blue-green algae, the morphologically and biochemically more complicated nitrogen-fixing forms are not found at temperatures as high as non-nitrogen-fixers (*31*).

Are Life Processes Faster at High Temperatures?

One of the key arguments against the concept of vitalism was that living organisms obeyed the Arrhenius equation relating reaction rate to temperature. However, a point which seems to have been overlooked throughout the history of kinetic biology (*43*) is that studies with the Arrhenius equation have been performed on single species at temperatures usually below the optimum for growth. If organisms do evolve so that their optima parallel their environmental temperature, as our data above suggest, then the relevant question is whether thermophiles function any faster at their optimum temperatures than mesophiles do at

their optima. The answer, from the limited available data, seems to be that thermophiles do not grow as rapidly as one would predict from the Arrhenius equation.

I have summarized the data on the growth rates of various bacteria at their *optimum temperatures* (Table 2). These data were obtained on organisms grown in rich media which probably permitted the maximum growth rate, and in each case the investigator attempted to provide sufficient aeration so that oxygen did not become a limiting factor. The data are plotted in the Arrhenius manner in Fig. 7; also included is the Arrhenius curve for the growth of *Escherichia coli* at various temperatures at and below its optimum (*44*). All of the points for the composite curve fall on a line which generally trends upward, but is much shallower than the curve for *E. coli*. Since Arrhenius curves for both mesophiles and thermophiles alone show not dissimilar slopes (*45*), we can assume that each individual species would give a slope similar to that of *E. coli*. However, since the slope of the collected data is much shallower, we see that thermophiles do not grow as fast at their optima as one would predict (*46*).

We can explain this result in several ways. First, growth may be less rapid than predicted because of continuous thermal destruction of sensitive molecules, so that the organism is always expending considerable energy in resynthesis. This seems unlikely on the basis of the earlier discussion. Second, the enzymes of thermophiles, because of their increased thermal stability, may be inherently less efficient, and hence less able to take advantage of the increased reaction rates of high-temperature environments (see the earlier discussion of aldolase). Third, there may be some inherent chemical (as opposed to biochemical) process limiting rapid growth. The rate of DNA unwinding provides an upper limit for growth rate (*47*), but from the equation of Freese and Freese (*47*) relating unwinding rate to temperature and molecular weight, and from the recent information on the molecular weight of bacterial DNA (*48*), we can conclude that there is more than enough time for DNA unwinding at any temperature where organisms grow. Thus, the foregoing discussion should dispel the notion that processes are inherently fast at high temperatures. Indeed, at very high temperatures, growth rate for algae even at the optimum is apparently low (*24*). Since the growth rate and thermal response are genetically fixed (*24*), this suggests that the organisms growing at the thermal limits have had to discard growth efficiency to survive at all.

Thermal Biology and the Origin and Evolution of Life

From the foregoing, we can see that there are definite thermal limits for the evolution of certain kinds of organisms. We also see that certain groups of organisms have evolved members that grow best at high temperature. Further, the temperature optima of organisms are quite stable genetic properties that are not subject to change by selection (*24, 36, 37, 45, 49*). Although temperature-sensitive mutants can be isolated for a variety of microorganisms, the thermal responses of these mutants differ from those of the parent by only a few degrees. To convert an organism with an optimum of 70°C to one with an optimum of 30°C, or the reverse, would probably require a large number of mutations. However, thermophilic species of blue-green algae and bacteria are related taxonomically to the mesophilic species of the same genera, and thus probably evolved from common ancestors.

It has been hypothesized (*21, 29, 50*) that the microorganisms of hot springs are relicts of primordial forms of life. Such a speculation does not seem unreasonable when we consider that evidence of hot-spring activity dates back to the Precambrian, and that certain rock formations (for example, the Gunflint chert, 2×10^9 years old, *51*), which probably have been formed in hot-spring deposits (*52*) teem with fossil microorganisms which resemble the Flexibacteria so common in thermal waters today. If organic matter, macromolecules, and primordial organisms arose at high temperatures, low-temperature forms might be derived from them by mutation and selection. In this process, changes in the kinetic properties of enzymes and of the protein-synthesizing machinery could be envisioned, so that a mesophile might sacrifice thermal stability in order to acquire an enzyme of more flexible conformation which would have a higher substrate affinity. In this way, mesophiles would be able to grow and function nearly as fast as thermophiles, even though they live at lower temperatures.

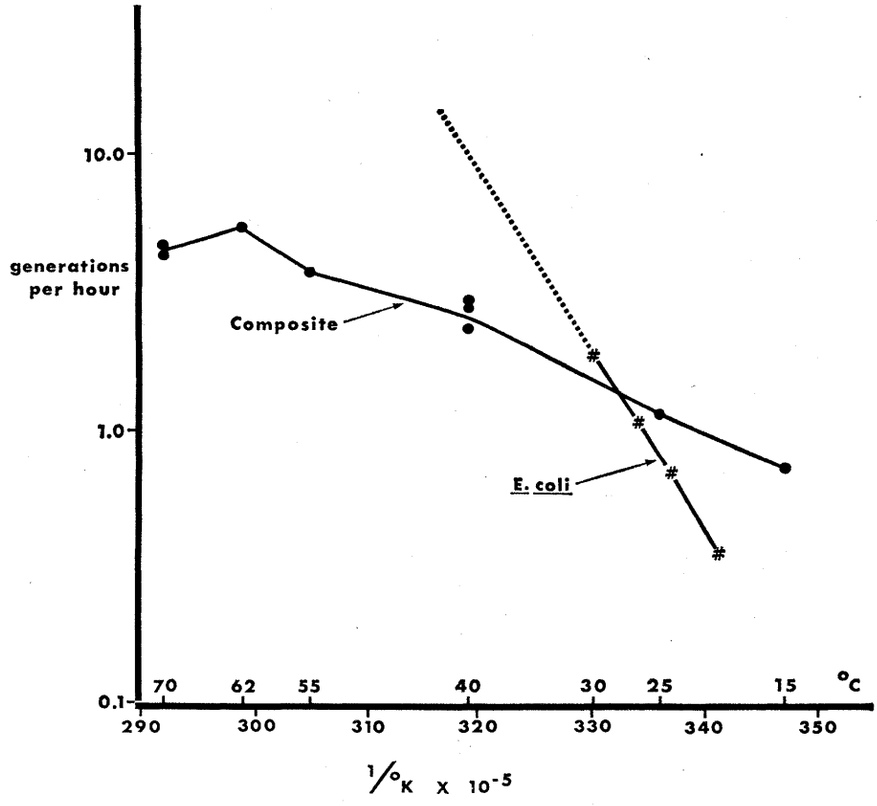


Fig. 7. The data from Table 2 graphed in the Arrhenius manner. The composite represents the growth rates of each organism at its optimum temperature. The data for *E. coli* are from reference (*44*).

Summary

The time is now ripe for a concerted attack on the evolutionary, ecological, and molecular aspects of life at high temperatures. Hot springs provide nearly ideal ecosystems for such study, since they are natural environments of great antiquity and relative constancy, where organisms have evolved to meet the environmental challenges of high temperatures. Even from our present limited knowledge, we can draw a number of conclusions.

1) The upper temperature for life as we know it has not yet been defined. At Yellowstone, some bacteria live and grow essentially at the boiling point. Since increased hydrostatic pressure may permit growth at even higher temperatures (*53*) there seems to be no reason why bacteria could not live in nature at any temperature where there is liquid water.

2) There is definitely an upper temperature for photosynthetic life, which seems to be at temperatures around 75°C for procaryotic algae. Eucaryotic microorganisms are restricted to lower temperatures (less than 60°C), and unicellular and multicellular animals to still lower temperatures (less than 50°C).

3) Our work in Yellowstone shows that the blue-green algae living at a given temperature are optimally adapted to that temperature, even if it is near the upper limit for algal growth. Thus, these algae are not merely lower temperature forms which have extended their range but are forms which have actually evolved so that their temperature optima resemble their environmental temperatures.

4) The molecular mechanism of thermophily is more likely to be related to the function and stability of cellular membranes than to the properties of specific macromolecules. In bacteria, where species have been studied with optima from 15° to 70°C, we may conclude from the limited data that each organism has many enzymes which are stable at the optimum temperature of the organism, but not at temperatures too much higher. This suggests that organisms do not evolve proteins which are much more stable than they need to be. Thus, there may be some advantage to the organism in synthesizing denaturable proteins. There may be a relation between protein denaturability and current ideas concerning induced fit and allosteric interactions of enzymes. The study of the kinetic properties of a single enzyme which is present in a wide variety of bacteria with differing temperature optima would provide an excellent contribution to our understanding of molecular evolution.

5) Although thermophiles grow somewhat faster at their optima than do mesophiles and psychrophiles at their optima, the increases are considerably less than would be predicted from the Arrhenius equation. This suggests that thermophiles, even though optimally adapted to their environments, are not able to make full use of their thermal environment.

6) In general, the temperature optima of organisms are not easily changed by mutation. On the other hand, thermophilic bacteria and algae are clearly related taxonomically to mesophilic species, and it seems reasonable that one group is derived from the other. One hypothesis has been that thermophilic organisms are relicts, and were more widely distributed on the earth in times when it was hotter than it is today. In this respect, it is interesting that fossil microorganisms in ancient rocks, such as the Gunflint Chert (2×10^9 years old) greatly resemble some of the Flexibacterales seen in hot springs today. It is not inconceivable that thermophilic microorganisms are related to primoridal forms which gave rise, through many mutations followed by selection, to mesophilic and psychrophilic forms.

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17. Plinius Secundus, Caius, ref. *4*, p. 354. "Patavinorum aquis caldis herbae virentes innascuntur, Pisanorum ranae, ad Vetulonius in Etruria non procul a mari pisces."
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24. R. W. Castenholz, *Symposium on the Environmental Requirements of Blue-Green Algae* (University of Washington, Seattle, in press).
25. T. D. Brock and M. L. Brock, unpublished observations.
26. I have quantitated this simple procedure in order to measure algal growth rate directly in nature. In one spring, I found a generation or doubling time of 24 hours, and in another of 12 hours, at temperatures of 70° to 72°C.
27. R. A. Lewin, private communication; S. Soriano and R. A. Lewin, *Ant. v. Leeuwenhoek* **31**, 66 (1965).
28. There is confusion concerning the taxonomic status of certain filamentous organisms living at high temperatures. Under the light microscope it is not possible to determine whether organisms around 1 μ in diameter have or do not have chlorophyll. Thus many species described from Yellowstone by Copeland [J. J. Copeland, *Ann. N.Y. Acad. Sci.* **36**, 1 (1936)], to be *Phormidium* and *Oscillatoria* are not algae. We have shown this by use of fluorescence microscopy, since chlorophyll can be detected in single filaments with much more sensitivity than by light microscopy. Further, by autoradiography we can show that these filamentous organisms do not fix CO_2 in the light (T. D. Brock, *Phycologia*, in press). This taxonomic confusion is still occurring in recent literature on Yellowstone material (J. E. Mann and H. F. Schlichting,

Trans. Amer. Microscop. Soc. **86**, 2 (1967).
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34. There is also a decreased biomass at temperatures below 55°C, possibly due to the activities of animal grazers [T. D. Brock, *Ecology* **48**, 566 (1967)].
35. T. D. Brock, *Nature* **214**, 822 (1967).
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The Process Values of University Research

A new research funding system is needed for federal support of the process values of university research.

James D. Carroll

In the 1960's tension has developed between support of university research by federal agencies for its "product values" and support for its "process values." Properly directed, this tension can be a creative one. Improperly directed, it can harm the university research enterprise in the United States.

The distinction between the process and the product values of university research should be recognized for purposes of analyzing federal university-research programs and formulating federal policies for the support of university research. In this article I analyze the differences between these values and suggest policy lines for the creative direction of the tension between them.

The Product Values

The product values are the values to federal agencies, to scientists, and to the public of the information produced. University research has four primary product values: the value of the information produced to agencies in the performance of their missions in defense, space, health, agriculture, and so on; the value of the information produced for the advancement of science as a worthwhile end in itself, and as a desirable cultural process; the value of the information produced to members of the public, particularly in civilian collective consumption sectors such as air supply, water supply, public health, public transportation, and public safety; and the value of the information produced as an element in economic growth.

Since publication of *Science—The Endless Frontier* in 1945 (*1*), the basic justification given for federal support of university research has been the potential or immediate value of the information produced. Year after year agency representatives have appeared before appropriations subcommittees and authorization and oversight committees expressing variations of the theme composed in its modern form by Vannevar Bush (*1*, p. 19):

> Basic research leads to new knowledge. It provides scientific capital. It creates the fund from which the practical applications of knowledge must be drawn. New prod-

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