to light regions in the film under polarized light.

Although discussion in this report has been limited to crystalline systems in which additives were used that are compatible with the polymer, we have also applied the technique to films of crystalline and noncrystalline polymers mixed with additives that are partially or entirely immiscible with the polymer. Distribution, size, and shape of labeled deposits are readily discernible, and such studies should yield valuable information in these kinds of systems as well.

Some decomposition of additive and host polymer due to radiation undoubtedly occurs. However, by the use of a probable "G value" for radiation decomposition, from the work of Tolbert and Lemmon (*8*), it can be calculated that decomposition would amount to less than 1 percent during the course of a typical experiment. To avoid any possible artifacts from such a source, our experiments were always carried out with recently purified additives and freshly prepared mixtures of additive and polymer.

J. D. MOYER
R. J. OCHS

W. R. Grace & Co.,
Washington Research Center,
Clarksville, Maryland

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17 September 1963

Upper Temperature Limit of Life

Abstract. *Samples of microorganisms from the hot springs of Yellowstone National Park have been collected and tested for the ability to utilize radioactive phosphorus. No evidence for growth was found above 73°C.*

Survival of organisms under extreme conditions may depend on isolation of their internal environment from inordinate salt concentrations, *p*H, or even pressure. However, there would be no defense against high temperatures unless the laws of thermodynamics were violated or other sources of energy were utilized. If cells were able to grow at almost boiling water temperatures, it is more likely that the cells would be at the local ambient temperature. The biophysics and biochemistry of such organisms would then be extremely unusual.

The highest temperatures on the surface of the earth (other than volcanoes) are found in the hot spring areas in Yellowstone National Park and also in Japan, New Zealand, and Iceland. Numerous reports of algae and bacteria found in these springs have been published during the last century. Many different people have claimed that these microorganisms were growing at temperatures as high as 89°C (*1*). In the laboratory, however, the highest growth temperatures which have been confirmed are 72° to 75°C (*2*). These experiments might suggest a maximum temperature for growth, and it is therefore of interest to use a metabolic test for growth of organisms reported in the hot springs.

All work was performed at Yellowstone National Park, Wyoming. The area of the lower geyser basin of the Firehole River was examined. Temperature measurements were made with thermistor probes. The highest temperature at which obvious algal mats were found was 73°C. All hot springs and their run-offs above 73°C are perfectly clear. Three springs along White Creek were studied in greater detail. In all springs there was strong convection, and the surface temperature was within 1°C of that at 10-cm depth. Samples were taken from the center of the pools, along with material scraped from the pool walls. Samples (100 ml) were sealed in sterile 1-liter polypropylene bottles containing 150 μc P^{32} as carrier-free H_3PO_4. These bottles were then incubated by floating them in the center of the pools where the samples were collected, and duplicate samples were placed at a single (cooler) point in the run-off of the spring. Other 100-ml samples containing algal mats were collected at that same point in the run-off and treated in a similar manner, being incubated at the point of collection and duplicates in the center of the hot pool.

Three pools were used with temperatures as follows. Pool No. 1 was 69.5°C at the center and 57°C in the run-off. Pool No. 2 was 93°C in the center and 72°C in the run-off. Pool No. 3 was 86°C in the center and 73°C in the run-off.

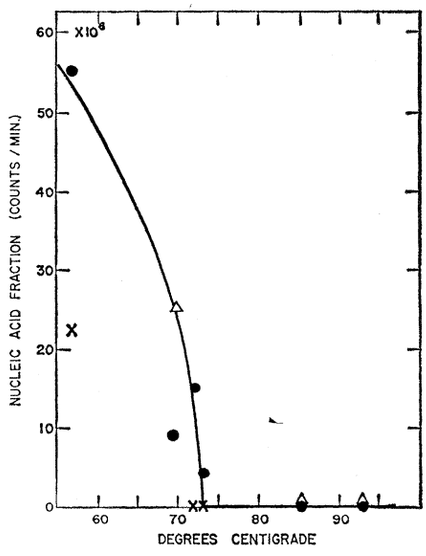

Fig. 1. Incorporation of radioactive phosphorus into nucleic acid at high temperatures. (Circles) Samples incubated at the same temperature as collected; (crosses) samples collected at pool center, incubated in the cooler run-off; (triangles) samples collected in run-off, incubated in hotter pool center.

The boiling point of water at the elevation of Yellowstone Park is 93°C.

Samples were incubated for 48 hours. Cells and other particulate material were removed by centrifugation and washed with 20 ml of tap water. The pellet was then extracted with 5 ml of 5 per cent trichloroacetic acid (TCA) in the cold. After centrifugation, the pellet was treated with 5 ml of 5 percent TCA in a boiling water bath for 20 minutes. The suspension was then chilled in ice and the residue was removed by centrifugation. The residue was resuspended in 2 ml of concentrated HCl. Aliquots of all samples were plated on stainless-steel planchets and evaporated to dryness. The radioactivity determinations were made with an end-window Geiger-Muller tube and scaler.

Any radioactive phosphorus which had been incorporated into the nucleic acids of growing cells would have been extracted in the fraction soluble in hot TCA. This fraction was therefore used as a measure of the growth or metabolism of the cells at each temperature. The results are shown in Fig. 1. It is clear that no evidence for active growth was observed above 73°C.

The explanation of the temperature limitation is unknown. Present concepts

of molecular biology would suggest that destruction of proteins, DNA, or RNA might be the cause. Koffler's studies (*3*) indicated that "thermophilic" forms were able to evolve with proteins that were more thermostable than similar proteins from mesophilic forms. Thermal studies in vitro of "soluble" RNA (*4*) revealed a maximum temperature for acceptance of amino acids about 75°C. The "melting apart" of the two strands of DNA at elevated temperatures has also been widely reported. The "melting temperature" has been shown to be characteristic for each species of DNA, depending primarily on the salt concentration (*5*) and secondarily on the guanosine-cytosine content of the sample (*6*). Although the internal salt concentration of thermophilic organisms is not known, it would be expected that the melting temperature of the DNA would be in the range of 70° to 90°C (*7*).

Although the evidence is scanty, a plausible explanation for a maximum temperature is the limitation of amino acid acceptance by soluble RNA. Whatever its molecular basis, it is clear that there is a maximum temperature for active life processes. The earlier ecological reports which have been widely quoted must therefore be reinterpreted as survival without metabolism. The limiting factors which prevent life forms as we know them from evolving at boiling water temperatures is worthy of further research (*8*).

ELLIS S. KEMPNER
National Institute of Arthritis and Metabolic Diseases, Bethesda 14, Maryland

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Red Blood Cells: Change in Shape in Capillaries

Abstract. *Evidence obtained from high-speed cinephotography of the microcirculation in the mesentery of the dog shows that the shape of the red blood cell is changed during its flow through capillaries from a biconcave disk to a paraboloid with a hollow bell-like center. The degree of deformation is dependent upon the velocity of flow in the capillaries. Although the effect of change in shape on the surface area of the cell is uncertain, a larger portion of the surface is brought into closer proximity to the capillary wall than in a cell that is in the form of a biconcave disk. Thus, the change in shape may have functional significance related to the exchange of respiratory gases.*

The relatively high plasticity of the mammalian red blood cell has been generally recognized. Its membrane, though apparently not highly elastic, is capable of deformation without irreversible alterations (*1*). The viscosity of the interior of the cell is probably about 30 times that of water at 38°C; this approximates the viscosity of olive oil at the same temperature (*2*). Thus the cell appears to have elastic and viscous characteristics which permit alterations in shape with the application of appropriate forces.

Although velocities of flow are higher in larger vessels, the red blood cell is subjected to a greater force that tends to distort its shape in its passage through the capillaries. The biconcave disk, as it enters a capillary, tends to be oriented with its polar axis parallel to the direction of flow, and the equatorial diameter of the disk approximates the diameter of the capillary. From measurements made with high-speed cinephotography in anesthetized dogs as described by Bloch (*3*), the velocities of red cells in mesenteric capillaries vary from 0.8 to 2 mm/sec. Thus if the plasma layer adjacent to the capillary wall is essentially stationary and the highest velocity of the plasma stream (parabolic flow) is in the center of the capillary (*4*), the center of the biconcave disk is subjected to forces acting in the direction of flow, while the peripheral edge of the cell is hindered in its forward movement by the essentially stationary ring of plasma adjacent to the luminal surface of the capillary. Such forces acting upon a deformable biconcave disk would be expected to cause it to approximate the parabolic front of the fluid system.

Changes in shape of red blood cells in vivo in animals other than the dog have been described. Palmer (*5*) in his studies of the microcirculation in the pancreas and mesentery of the rat, commented on the distortion of red cells when single rows of cells, moving through narrow vessels, are observed. He states, "the leading surface is markedly convex and the trailing surface is less convex or even concave." His photographs demonstrate this transformation when the cells are not too closely packed. With high-speed cinephotography, Bloch (*3*) studied the microcirculation in the mesenteries of frogs and rats. The published reproductions of his photographs show a tendency for the leading surface of frog erythrocytes in narrow vessels to be convex and the trailing surface concave. Brånemark and Lindström (*6*) have recently described various changes in the shape of the red blood cell in narrow circulatory passageways of modified ear chambers in rabbits. Among the changes which they describe are successive transformations from biconcave disks to contours resembling jellyfish, torpedoes, and bullets.

Observation and recording of the behavior of erythrocytes in capillaries were made possible through the use of high-speed cinephotography. The mes-

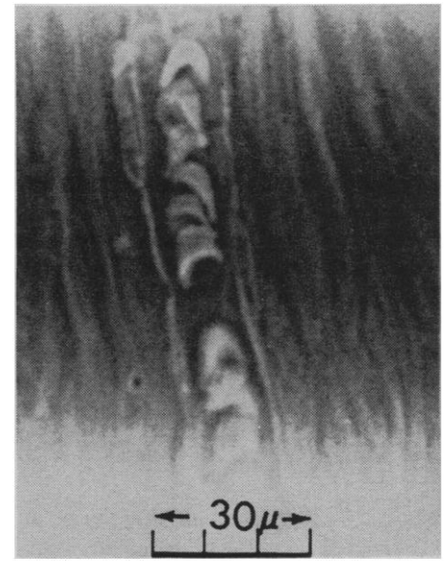

Fig. 1. Reproduction from one frame of film taken at 3200 frames per second of red blood cells passing through a capillary. Each division of the scale represents 10 microns.