marked E is a doubled curve. Figure 3B is an enlarged view of the outlined box of Fig. 3A. The smaller inner curve (Fig. 3B) is the received echo before acid treatment, and the larger outer curve is the echo after acid treatment. The picture was obtained by double exposure. The scope image was adjusted so that the echo from the transducer tip, AE, in the second exposure was superimposed on that of the first exposure.

If there were no effect on the echogram due to the surface demineralization, both images would appear as one. Since there are two images, it is clear that the change in the surface condition did modify the wave shape of the echo. The picture in Fig. 3 was the result after a 10-minute exposure of the tooth surface to dilute acid.

Although the change in shape is small, it is easily detected. Changes due to additional exposure of the tooth surface to the acid caused the echogram to change in the same sense, an indication of a definite correlation between the exposure to acid and the response in the wave shape. It is presumed that a means for measuring the state of mineralization of the tooth surface by ultrasound has been established.

The change in the wave shape of the ultrasonic echo may be due to a change in the sonic propagation characteristics of the surface, as suggested by Gilmore's work (*8*). Or it may be due to the observed changes in the surface structure or even possibly to the actual mechanical displacement of the tooth surface. The effect is probably attributable to all three causes but it is not known which is dominant.

An instrument system has been designed to reduce the observed effect to quantitative terms (*12*). In this system the complex ultrasonic wave form is reduced to digital form and the digital signals are stored in the core memory of a computer for transfer to magnetic tape. The digital transformation process employs a sampling process and takes advantage of the fact that the echo pattern of the ultrasonic system changes very slowly. In the instrument system the design calls for a pulse sampling rate of 10 khz. The part of the wave under investigation can be adequately represented by 100 to 200 points. In 1 second between 50 and 100 repeated observations can be made of each sample; thus signal averaging may be employed. Since the data are in digital form it will be possible to process the data on-line, with all available theory being used to extract information. The instrument system is being built and should be operable by 1 October 1970.

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Limits of Microbial Existence: Temperature and pH

Abstract. *A microscopic survey made to detect the presence of bacteria in hot springs of varying temperature and* pH *characteristics revealed that in neutral and alkaline hot springs bacteria are found at temperatures up to the boiling point of water (92° to 100°C, depending on the altitude). In hot springs of increasing acidity the upper temperature limit at which bacteria are found decreases; at* pH *2 to 3 the upper temperature limit is 75° to 80°C. Bacteria have thus been able to evolve with the ability to grow at either high temperature or high acidity, but not at both high temperature and high acidity. These results suggest that there are physicochemical limitations of the environment beyond which life is impossible.*

Our earlier studies (*1–3*) have shown that in neutral or alkaline hot springs bacteria live and grow in boiling water. Virtually every neutral or alkaline hot spring examined at Yellowstone National Park showed fairly high population densities, and measured growth rates (*3*) were rapid. Since at the altitude of Yellowstone Park water boils at 92.5° to 93°C, even superheated springs have temperatures which rarely exceed 95°C. Recent studies in New Zealand and Iceland, where the boiling springs are at lower altitudes and have temperatures of 99° to 101°C, have shown that at these locations also virtually every spring in the neutral and alkaline *p*H range has high populations of bacteria.

In acid hot springs, on the other hand, bacteria often appear to be absent (*4*). Only at temperatures around 60° to 65°C do macroscopically visible accumulations of bacteria appear in acid springs, whereas in neutral and alkaline springs such accumulations are seen at 85° to 90°C (*2*). It seemed, therefore, that acid might add an additional environmental stress which could prevent bacteria from evolving with the ability to grow at the highest temperatures. To document this possibility in some detail, we have surveyed for the presence of bacteria a large number of hot springs of various temperatures and *p*H characteristics in Yellowstone Park, in other thermal areas of the western United States, in New Zealand, and in Iceland. The results of this survey show clearly that as the environmental *p*H decreases, the upper temperature limit at which bacteria are observed also decreases. In addition to a general survey of springs, we have also studied the thermal gradients of several acid springs, in which bacteria appeared to be absent from the source, to determine the highest temperature at which bacteria could be found. These studies show that there is nothing intrinsically unfavorable for bacterial growth in acid springs. In fact, we have detected the presence of a number of bacteria that actually require acid conditions for growth.

Almost 300 individual springs were examined for the presence of bacteria. Of these, 163 were in Yellowstone National Park, 21 were in other parts of the western United States (Lassen Volcanic National Park, California; The Geysers, California; Steamboat Springs, Nevada; and Beowawe, Nevada), 68 were in the central volcanic region of the North Island of New Zealand, 40 were in Iceland, and 2 were in Japan.

Temperatures were measured with thermistors (Yellow Springs Instrument Co.) checked occasionally against mercury thermometers. The pH values were measured in the field by means of a pH meter (Orion) and a combination electrode (Corning). If the pH was not measured immediately upon collection, the water was taken in a plastic bottle which was filled completely to the top, and the pH value was read within a few hours. Checks showed that pH values did not change significantly for at least a few hours when water stood in filled bottles.

Assessment of the presence or absence of bacteria was made with a phase microscope. Experience has shown that the filamentous and rod-shaped bacteria living in hot springs exist primarily attached to mineral particles or to the siliceous walls of the springs, and, when present, are usually seen in quite large numbers. The bacteria are also sloughed into the water and will attach to glass microscope slides immersed in the water, upon which surfaces they will grow and form dense colonies, usually within a week. When possible, the presence of bacteria was assessed by phase microscopic examination of pebbles or samples of rock ripped from the walls of the spring. In some cases the structure of the spring made it impossible or unsafe to remove rock samples. In some of these cases 100-ml samples of water were taken and passed through membrane filters; the residue from the filter was suspended in a small amount of water and examined under the phase microscope for the presence of bacteria. In other cases microscope slides were immersed in the springs, left for a week, removed, and examined. This last method, probably the most sensitive for detecting bacteria, was also used to confirm the presence or absence of bacteria in a number of springs that were of special interest.

In virtually all cases, detection of the presence of bacteria was unequivocal; that is, either a large number of cells were present so that some were seen in every microscope field, or none were present even after the examination of 30 to 50 fields. In a few equivocal cases (less than 1 percent of the samples) only a rare bacterial cell was present. In these cases the bacteria were never attached to particles but were free-living; presumably these could be cells that had recently fallen into the water. Since the springs from which these samples were taken were not of such temperature-pH characteristics that a clear-cut decision of their bacterial content would alter the overall picture, the results from these springs are not included in the data. The main limitation of our survey is that it will probably not detect very small organisms or ones with irregular or highly unusual morphology, since such organisms would not necessarily be distinguished from mineral particles. However, even spherical organisms such as cocci or mycoplasmas can be distinguished from mineral particles with the phase microscope since the refractilities of the latter are quite different from those of microbial cells. From studies with immersion slides, which permit superior microscopy, we have found that cocci and coccobacilli are absent from hot springs, although some springs have spherical forms resembling mycoplasmas or protoplasts.

The data, summarized in Fig. 1, indicate that at pH values above 4 bacteria were found in springs at 90°C or higher, but at pH values below 4 the upper temperature limit at which bacteria were found progressively decreases. At pH values below 3 an upper temperature limit beyond which no bacteria were found occurs in the range from 75° to 80°C. Unfortunately, not many springs exist with pH values between 4 and 6, so that it was difficult to define precisely the upper temperature boundaries in this region. At pH values of 7 or higher bacteria are found in boiling water, even at temperatures of 99° to 100°C. A few springs in this neutral to alkaline pH range were devoid of bacteria, probably because some other feature of the en-

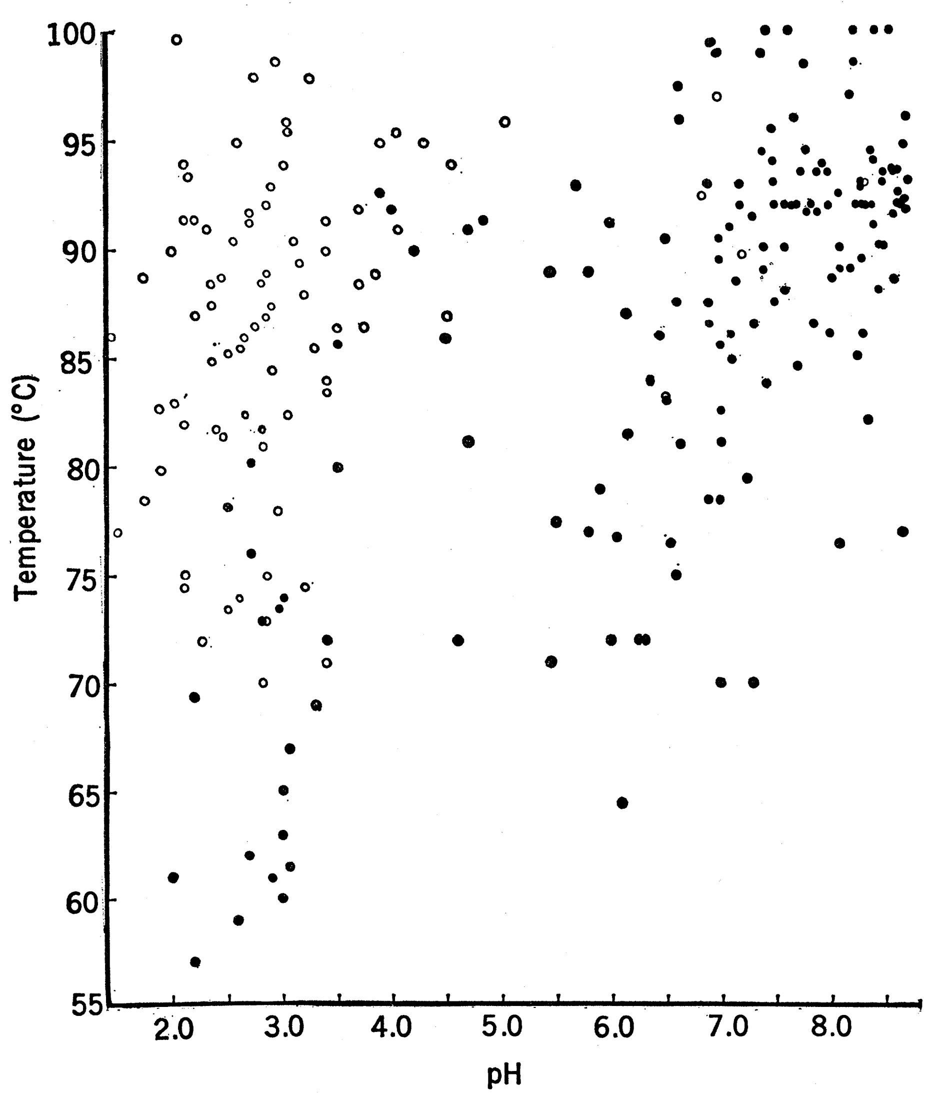

Fig. 1. Presence of bacteria in hot springs of various temperature and pH characteristics. Each dot represents the results for a single spring. (Open circles) Bacteria absent; (solid circles) bacteria present. Data for Icelandic studies or for springs with pH greater than 8.5 are not given.

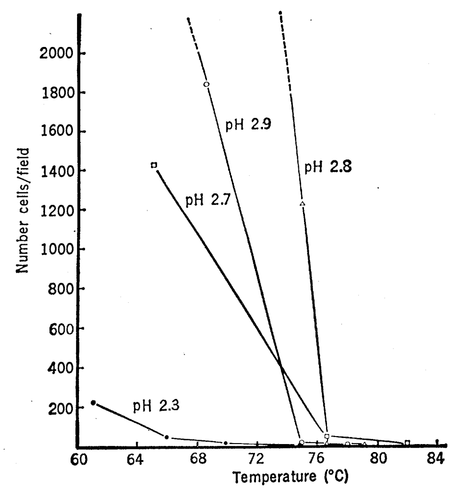

Fig. 2. Quantification of bacterial numbers at different temperatures along the thermal gradients of four acid springs. Microscope slides were immersed at different temperatures for a week; then they were removed and bacterial numbers per microscope field (3.7×10^4 μm^2) were counted. The dotted lines extend toward approximate values for slides that had too many bacteria for precise counting.

vironment was unfavorable for growth.

For the acid high-temperature springs, from which bacteria were always absent, it is important to ascertain that the waters would be favorable for bacterial growth upon cooling. This could most easily be done by observing the presence or absence of bacteria at various temperatures along the outflow channels of such acid springs, since this would provide a series of temperatures all in water of the same chemical composition. (There is no change in *p*H of the water upon cooling.) When such studies were made, it was found that bacteria did develop in these cooler waters, an indication that the water was indeed favorable for bacterial growth, provided that the temperature was not too high. The upper temperature limits observed along these thermal gradients were 70° to 75°C, which is the same as the upper temperature limit defined by studies of the whole group of springs (Fig. 1).

Quantitative studies on the rate of bacterial growth were performed at different temperatures along the thermal gradients of a few springs by immersing slides in the water for defined periods and then counting the number of bacteria per unit area of slide. These data (Fig. 2) also reveal that the upper temperature limit for bacterial growth in waters of *p*H 2 to 3 is 70° to 75°C. A further indication that low *p*H is not inherently limiting for bacterial growth is the fact that a number of obligately acidophilic bacteria which are also moderate thermophiles have been obtained in laboratory culture. Kaplan (*5*) and Schwartz and Schwartz (*6*) obtained cultures of acidophilic thiobacilli able to grow at temperatures up to 55°C, and Brierley (*7*) has obtained ferrobacilli of similar character. Uchino and Doi (*8*) isolated several acidophilic, thermophilic spore-forming bacilli, and we have confirmed and extended these observations (*9*).

High acidity also limits the upper temperature limit at which algae are found. Thus, although blue-green algae are found in neutral and alkaline hot springs at temperatures up to 73° to 75°C (*1, 10*), in acid waters the upper temperature limit at which an alga (*Cyanidium caldarium*) is found is 55° to 56°C (*2, 11*).

It thus seems as if high acidity may add an additional environmental stress that makes microbial growth at very high temperatures impossible. These results are of considerable interest in defining the physicochemical limits within which life is possible. It would now be of considerable interest to examine the stability to acid and high temperature of various kinds of cellular constituents to see which types are most likely to be labile. It is most likely that resistance to high acidity is associated with the impermeability of the cell to hydrogen ions (*2*), thus focusing attention on the cell membrane.

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Nuclear Sexing in a Population of Congolese Metropolitan Newborns

Abstract. *In a study of 5631 Congolese newborn children, the incidence of numerical X-chromosomal anomalies was the same as was previously reported in Europe and in North America.*

An extensive survey on the Congolese newborn was carried out between March and June 1969 at the Hôpital Général des Congolais in Kinshasa, in which hospital 80 to 120 births are registered per day (about 55 percent of all births in Kinshasa).

The survey was intended to provide a broad insight into problems presently encountered in newborn children in the large metropolitan city of Kinshasa (nearly 1 million inhabitants). Substantial information about various aspects of the mothers' and children's health, including some genetic and social aspects, has been obtained.

Nuclear sexing of buccal mucosa cells was performed on all liveborn children and all stillborn fetuses of at least 1000 g with the exception of the children and fetuses born every 6th day. The preparations were stained with aceto-orcein (*1*) and interpreted by two different investigators. All doubtful or abnormal smears were repeated. In every child, 500 cells were counted on two different slides. The total number of newborns examined was 5,631. Eight of the 2,834 males, all liveborn, had chromatin-positive buccal smears (2.8 per thousand). Chromosome analysis of peripheral blood