A RELATIONSHIP BETWEEN MULTIPLE TEMPERATURE OPTIMA FOR BIOLOGICAL SYSTEMS AND THE PROPERTIES OF WATER

C. H. OPPENHEIMER[1] AND W. DROST-HANSEN

Research Center, Pan American Petroleum Corporation, Tulsa, Oklahoma

Received for publication November 16, 1959

It has been suggested (Drost-Hansen and Neill, 1955; Lavergne and Drost-Hansen, 1956) that a number of more or less abrupt changes in the properties of water and aqueous solutions occur when the temperature is increased from 0 to 60 C. These changes or "kinks" occur within a rather narrow temperature range (±2 C) near 15, 30, 45, and 60 C, respectively. The kinks are believed to be caused by structural changes in the water. In a previous note (Drost-Hansen, 1956) it was postulated that these structural changes influence the behavior or activity of biological systems. It is believed that optimal conditions for a complex physiological activity (such as, for instance, growth) will occur somewhere near the middle of the interval between two consecutive kinks. In the middle of the interval, the liquid is structurally most stable for small temperature variations, and thus offers at this temperature the most stable conditions for biological activity. The exact temperature of the optimum will be determined by the simultaneous effects of temperature on the rate phenomena occurring in the system (compare Arrhenius' law and Eyring's rate theory) and the postulated structural changes. Abrupt changes for small temperature variations as well as minima for physiological activity will occur at the temperature near the kinks, where the structure of the water changes rapidly with small changes in the temperature. As an example, Mitchell and Houlahan (1946) reported optima for the growth of *Neurospora crassa* near 23 and 37 C, and growth minima near 15, 30, and 45 C. These findings are in excellent agreement with the notion that the structural changes in water directly influence the activities of living organisms.

In a recent experiment to determine the temperature optima for the growth of a sulfate-reducing bacteria, we have observed no less than three temperature optima at 11, 25 and 39 C, and minima near 16, 31, and 43 C. The data presented here are qualitative only. They were obtained with the use of a specially designed polythermostat which allowed the cultures to be grown simultaneously at the different temperatures. Because the phenomenon of multiple growth optima has since proved to be reproducible, the initial data are being presented at this time.

MATERIALS AND METHODS

The preliminary data were obtained using a pure culture of an unnamed sulfate-reducing bacterium, probably a Clostridium which was being studied during corrosion experiments. The organism was obtained from a marine environment. It is an anaerobic, sporulating, motile rod which reduces sulfate and sulfite to sulfide.

The medium in which the growth experiments were conducted was prepared as follows: peptone (Difco), 1.0 g; yeast extract (Difco), 1.0 g; Na-lactate (60 per cent), 4 ml; Na_2SO_4,0.5 g; Na_2SO_3, 0.2 g; distilled water, 100 ml; pH adjusted to 7.0 with NaOH. The sterile medium is clear and the growth of the organism produced a visible turbidity which was used as a measure of activity. The inoculated medium contained approximately 150 active bacteria per ml.

In microbiological research it is often necessary to measure accurately optimal temperatures for growth and other physiological activities. The measurements should preferably be made at the same time to minimize other, external variables. Several devices have been reported which will provide simultaneously a number of different temperatures (Henson, 1957; Herter, 1943; Halldal and Stacy French 1956). However, these devices are not easily adapted for general microbiological research. For this reason, a versatile and easily constructed polythermostat was designed. The thermostat consists of an aluminum bar (4 by 4 by 30 in.) in which is produced a temperature gradient. One end of the bar is heated by three "Ungar" soldering iron elements,

[1] Present address: Institute of Marine Science, University of Texas, Port Aransas, Texas.

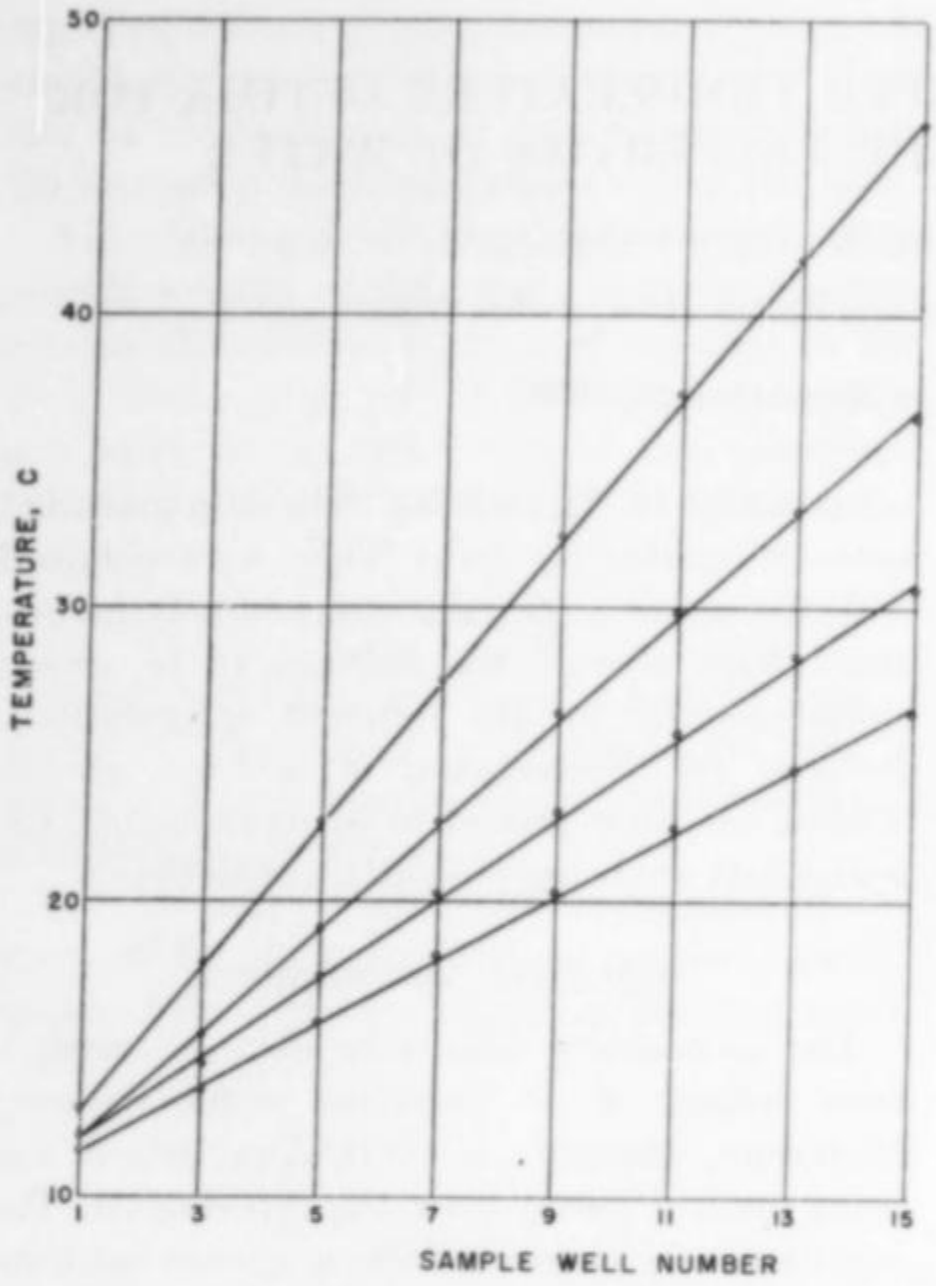

Figure 1. Distribution of temperature in the sample wells of a linear gradient polythermostat.

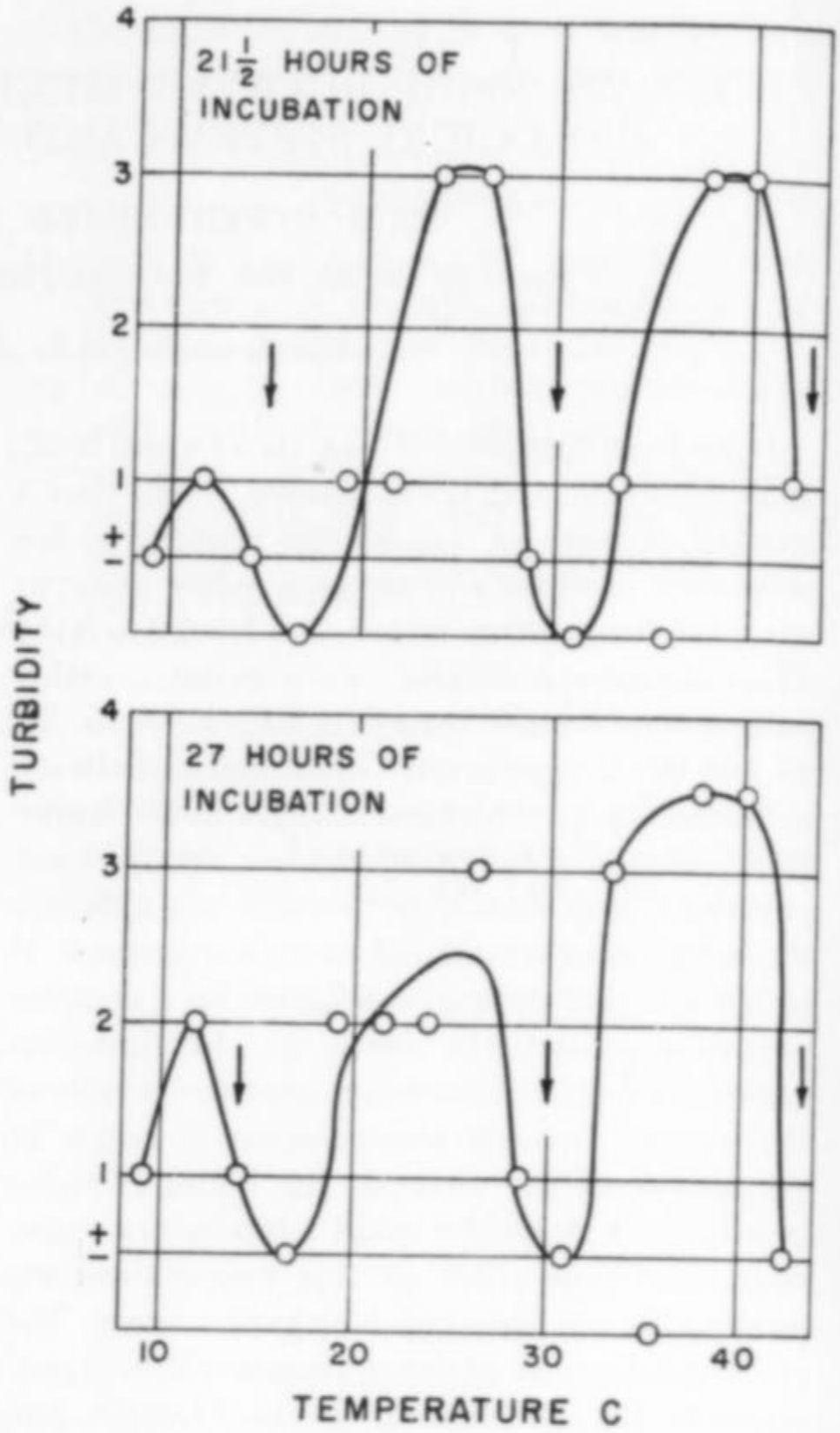

Figure 2. Growth of a sulfate-reducing bacterium at different temperatures as measured by turbidity.

inserted in three vertical wells across the end of the bar. The rate of heating is controlled by the use of a variable output transformer, preferably operated off a constant voltage line. The other end of the bar is cooled by cold water which is circulated through a simple network of channels close to the end of the bar. A thermoregulator located near the cold end of the bar controls a pump which circulates cold water. The bar is insulated with 2 in. of polystyrene foam on the sides, ends, and bottom. The top (working side) is insulated with two layers of flexible, ¼-in. polyurethane foam. The entire bar and insulation rests in a wooden box. Samples placed in vertical wells along the temperature gradient will be subjected to a number of different, constant temperatures. Fifteen such sample wells, ¾ in. in diameter and 3½ in. deep are situated at equidistant points (1⅝ in. apart) along the bar. Test tubes (16 mm diameter) or Thunberg tubes which fit loosely into the sample wells are used to contain the samples. The 4-in.-wide bar is large enough to accomodate two or three rows of sample wells.

The entire thermostat is agitated rapidly by a mechanical shaker. Because of the temperature gradient in the bar, a finite temperature difference exists between opposite sides (along the bar) of each sample well. The shaker device spins the test tubes in the sample wells. Thus, the effective temperature of each sample becomes an average of the (small) temperature difference of the opposite sides of the well. The temperature at different points along the bar is determined with thermistors or short-stemmed mercury thermometers situated in wells at regular intervals along the bar. Figure 1 illustrates the near linear temperature gradient between 10 C and a number of higher temperatures (in this particular experiment, the temperatures were measured in the sample wells). Over a period of several days the temperature variations at any point did not exceed ±0.1 C. It is expected that the tempera-

ture gradient along the bar may be regulated easily between 0.01 and 2 C per cm at any temperature between 0 and 125 C.

At the start of each experiment sterile Thunberg tubes were filled with inoculated medium and placed in the polythermostat. In the present series of experiments the rate of growth was estimated from visual observations of the turbidity as averaged by the two authors (in more recent experiments, which have confirmed quantitatively the preliminary results discussed here, the rate of growth has been determined by the use of a spectrophotometer).

RESULTS

Figure 2 shows the relative amount of growth as a function of temperature after 21½ and 27 hr growth. The minima for the growth of the organism (near 16, 31, and 43 C) are seen to coincide quite closely with the temperature of the kinks in the properties of water near 15, 30, and 45 C. The two higher temperature optima (near 25 and 39 C) are seen to coincide with the predicted temperature optima near 23 and 37 C. The temperature optimum below 15 C had not been predicted because of insufficient evidence for a kink below 15 C.

In replicate experiments, one or sometimes two of the optima may be absent. Also, two optima may not be clearly separated and the growth curve then takes the appearance of two superimposed distribution functions (with different maxima). We have since found that the experimental growth conditions are very critical and small changes in the medium composition or presence of residual detergent in the test tubes will influence the experimental results. When the cultures were allowed to incubate for 48 hr, maximum turbidity developed in all the tubes within the growth range and the effects of the structural changes in the water could no longer be detected. This fact is taken to indicate that the complete lack of growth near 35.5 C in the experiment shown in figure 2, is due to failure of inoculum to grow; in replicate experiments no anomalous behavior was noted at this temperature.

DISCUSSION

The agreement between the observed and predicted temperature optima and minima supports the theory that the structural changes in water and aqueous solutions exert a direct influence on biological phenomena. It should be noted that the presence of the small amount of ions and undissociated molecules in the culture medium does not influence the temperatures of the kinks (Drost-Hansen and Neill, 1955). In general, noticeable changes in the temperatures at which the kinks occur caused by the presence of ions have been noted only in a small number of aqueous solutions, mainly for the highly dissociated acids, and for a few other electrolytes, at concentrations above 1 to 5 molar. This is in agreement with the observation that only ion concentrations greater than approximately 1 molar significantly increase the "structural temperature" of water (Bockris, 1954).

A living organism normally has several metabolic pathways (provided the proper substrates are present) which can be used to obtain energy and to provide protoplasm and which are influenced directly by temperature. On the basis of our experiments, the hypothesis is offered that a single species of bacteria may have optimal activity at more than one temperature by utilizing different metabolic pathways. The more or less abrupt changes which occur in water will influence the selection and nature of the metabolic reactions associated with the growth (compare Campbell and Williams, 1953). Considering the evidence for Neurospora and the present results, it is suggested that the hypothesis presented here may have general validity and should be explored further.

The occurrence of the kinks and their apparent significance to the optimal temperatures of living organisms suggested the possibility that a living organism may have more than one optimal temperature for activity in the natural environment. If microorganisms in general have the ability to grow or be active optimally in different environments this may account for increase in activity at certain periods of the annual cycle corresponding to the predicted optimal temperature. It may also account for microbial activity at cold and warm latitudes, and suggests that the microorganisms which are optimally active in the polar environments may also be active in the tropical environments at temperatures between the kinks.

SUMMARY

A polythermostat has been developed which permits microbiological studies to be carried out

simultaneously at a number of different temperatures.

An unnamed, sulfate-reducing bacterium in pure culture, cultivated in minimal medium in the polythermostat, exhibited multiple temperature optima for growth at 11, 25, and 39 C, and minima near 16, 31, and 43 C.

REFERENCES

BOCKRIS, J. O'M. 1954 *Modern aspects of electrochemistry*, pp. 91–92. Butterworth & Co., London.

CAMPBELL, L. L., JR., AND WILLIAMS, O. B. 1953 The effects of temperature on the nutritional requirements of facultative and obligate thermophylic bacteria. J. Bacteriol., **65,** 141–145.

DROST-HANSEN, W. 1956 Temperature anomalies and biological temperature optima in the process of evolution. Naturwissenschaften, **43**, 512.

DROST-HANSEN, W. AND NEILL, H. W. 1955 Temperature anomalies in the properties of liquid water. Phys. Rev. (Abstr.), **100,** 1800.

HALLDAL, P. AND STACY FRENCH, C. 1956 The growth of algae in crossed gradients of light intensity and temperature. Carnegie Inst. Wash. Yearbook, **55,** 261–265.

HENSON, W. R. 1957 Temperature preferences of *Grylloblatta campodeiformis walker*. Nature, **179,** 637.

HERTER, K. 1943 Die Beziehungen zwischen der Ökologie und der Thermotaxis der Tiere. Biol. generalis, **17,** 243.

LAVERGNE, M. AND DROST-HANSEN, W. 1956 Discontinuities in slope of the temperature dependence of the thermal expansion of water. Naturwissenschaften, **43,** 511–512.

MITCHELL, H. K. AND HOULAHAN, M.D. 1946 Neurospora. IV. A temperature sensitive riboflavinless mutant. Am. J. Botany, **33,** 31–35.