TEMPERATURE-GRADIENT PLATES FOR GROWTH OF MICROORGANISMS

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Received for publication August 17, 1961

ABSTRACT

LANDMAN, OTTO E. (Fort Detrick, Frederick, Md.), HOWARD T. BAUSUM, AND THOMAS S. MATNEY. Temperature-gradient plates for growth of microorganisms. J. Bacteriol. **83**:463–469. 1962.—Different temperature-gradient plates have been devised for the study of microbial growth on solid media through continuous temperature ranges or in liquid media at finely graded temperatures. All plates are made of heavy-gauge aluminum; heat supplied at one end is dissipated along the length of the metal so that a gradient is produced. The shape and range of the gradient depends on the amount of heat supplied, the insulation, the ambient temperature, and other factors. Differences of 0.2 C in temperature sensitivity between bacterial strains can be detected. The plates are simple to construct and operate. The dimensions of the aluminum, the mode of temperature measurement, and the method of heating may all be modified without diminishing the basic utility of the device.

A sharp growth front develops at the maximal temperature of growth of bacteria. In most strains, all bacteria below the front form colonies and all bacteria above the front are killed, except for a few temperature-resistant mutants.

In studying the response of bacteria or enzymes to incubation temperature, a set of accurately controllable incubating devices is often required. In response to this need, we have developed a series of instruments which provide continuous temperature gradients on solid media or in a series of tubes with liquids at finely graded temperatures. We have named these instruments "temperature-gradient plates."

Previous to our first report on a temperature-gradient plate (Bausum, Landman, and Matney, 1960), Halldahl and French (1958) had used a temperature gradient on solid medium to study the effect of light on algal growth at different temperatures. More recently, Oppenheimer and Drost-Hansen (1960), using the same principles, described a device which provides graded temperature increments for a series of broth cultures.

The purpose of this paper is to describe four temperature-gradient plates which were developed for a variety of uses in this laboratory and which have proved to be extremely convenient in bacteriological experimentation. Our plates differ from those described by the other authors in many details of construction and in the elimination of water cooling.

APPARATUS AND METHODS

Each of the temperature-gradient plates consists of a heavy, flat piece of rolled aluminum with a heating element applied to one end. The heat supplied by the heating element is dissipated along the length of the aluminum so that a temperature gradient is established. The temperature at the hot end depends on the power input. This is usually controlled by a 150-w constant-voltage transformer (Sola Electric Co., Chicago, Ill.) and a rheostat connected in series. A "Thermistemp" regulating unit (Yellow Springs Instrument Co., Yellow Springs, Ohio), with its sensing element applied at the hot end of the temperature-gradient plate, may also be used. Indeed, preliminary experiments suggest that the latter method provides improved temperature control.

Wide-lane plate. The wide-lane plate (Fig. 1) was made from a solid block of aluminum, 86.4 by 28.2 by 3.8 cm. Five 75.3 by 5.1 by 2.5 cm longitudinal channels (to contain agar medium) have been machined into the block, and a 10-cm-wide strip at one end has been cut to a thickness of 1.3 cm to provide an area where the heating unit may be affixed. The heating unit itself has been made detachable, so that it need not be autoclaved with the rest of the gradient plate. It consists of two 3.8 by 26.7 cm 250-w Chromalox strip heaters (Edwin L. Wiegand Co., Pittsburgh, Pa.), mounted in an aluminum casing so that the

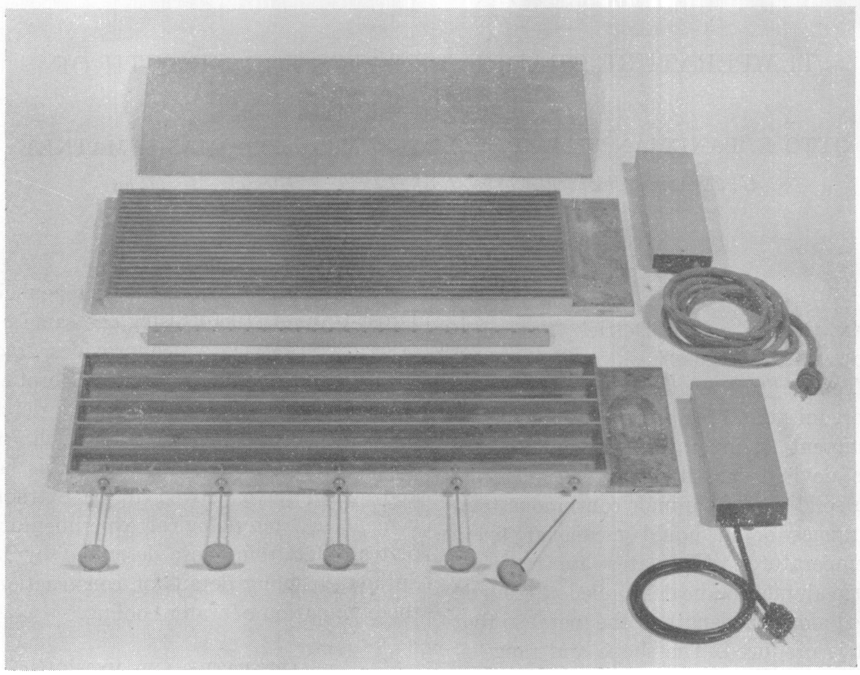

FIG. 1. *Wide-lane temperature gradient plate (foreground), narrow-lane plate, detachable heating units, metal-stemmed thermometers, and an aluminum lid used with the wide-lane plate. With the 21-lane plate, a Transite lid with holes for insertion of small glass thermometers is used (not shown). The channel widths of the two plates are, respectively, 5.1 and 0.8 cm.*

heaters and the gradient plate are in direct contact when the heating unit is snapped into place by means of its spring clamps. An 0.6-cm-thick, 75.6 by 28.5 cm aluminum lid covers the lanes to insure sterility and reduce evaporation. At 16.5-cm intervals along the lengthwise side wall of the plate, dial-type metal stem thermometers are inserted through bushings with rubber grommets into the agar of the first lane. The ends of the thermometers lie just below the surface of the agar and thus give an accurate measure of the temperature which is experienced by the cells on the agar surface.

In a typical experiment with this plate, approximately the following procedure is used. After inserting the alcohol-sterilized thermometers into their sleeves, melted agar medium of the desired type is poured into the lanes; 500 ml per lane give a layer about 1.3 cm thick. The lid, wrapped in turkish toweling, is placed over the plate, and the agar is allowed to solidify at room temperature. The gradient plate, the thermometers, the agar, and the wrapped lid are kept sterile throughout. The heating element is now clamped on, the plate is placed in the desired environment (usually a constant temperature room, cold room, or incubator), and the rheostat control is adjusted to the setting appropriate to the desired temperature. When steady thermometer readings indicate that equilibrium has been reached, the lanes are inoculated. This is sometimes done by placing a few milliliters of the desired concentration of cells at one end of each lane, lifting that end of the plate, and allowing the fluid to run to the other end. Excess fluid is then removed with a pipette. Because it is rapid, this method of inoculation minimizes the unavoidable drop in temperature that occurs when the lid is lifted for inoculation. Unfortunately, it is difficult to get quantitatively reproducible inoculation by this method. When colony counts are required, the lanes are therefore inoculated by spreading known volumes of suspensions on measured lengths of lane. Since at least 30 min are required to re-establish temperature equilibrium after this procedure, other methods must be used to study those particularly rapid changes in growth response which occur at high temperatures (Bausum, Matney, and Landman, 1961). The agar-layer technique employed for phage work can be used in experiments with the gradient plate if the plate is heated after the agar layer has solidified. Equilibration can be hastened by heating the plate with a Bunsen burner.

In work with the temperature-gradient plate, both desiccation and condensation are problems. Each lane is in effect a condenser: water evaporates at the warm end and condenses at the cooler end on the lid. Several measures may be taken to reduce condensation or its effects. For one, the gradient plate can be inverted during incubation, to prevent dripping of water from the lid onto the agar surface, and turkish toweling is then used to soak up the condensed water. Further, the temperature differential between the agar and the lid may be reduced by using lids made of insulating material such as Transite (Johns-Manville, New York, N. Y.), by insulating the aluminum lids with sheets of Transite, or by heating the lid to a temperature corresponding to or higher than that of the plate. By using a heated lid and a water-tight grease seal between the plate and its lid, both condensation and desiccation are minimized and no cracks will appear in the agar, even if incubation is extended over several days.

The wide-lane gradient plate has been useful in experiments in which colony counts or phage plaque counts in different temperature ranges were required, in experiments in which large populations were screened for temperature mutants, and in experiments in which bacteria impinged on 5.1-cm diameter Millipore filters were transiently exposed to the gradient.

Plate with 21 lanes, 0.8 cm wide. This gradient plate was made from a solid aluminum block, 83.8 by 30.8 by 3.2 cm, and is also shown in Fig. 1. The 71.1-cm-long, 0.8-cm-wide, 1.9-cm-deep parallel longitudinal channels were milled into the plate with a wheel cutter. Each lane, when filled to a depth of 1.3 cm, holds about 75 ml of medium. The heating unit used for this plate is interchangeable with that employed with the 5-lane plate. Comparison of temperature-limited growth in lanes containing identical media and inocula have shown that, in contrast to the 5-lane plate, the outside lanes on either side of the 21-lane plate equilibrate at a slightly lower temperature than the remainder of the lanes. To get correct temperature measurements for these 19 center lanes, thermometers would have to be inserted at least as far in as the second lane. To circumvent the necessity for an elaborate sleeve arrangement, thermometers have therefore been introduced through holes in the lid in experiments with this plate. The 21-lane plate is inoculated by the pipetting method described. This plate has proved useful in experiments in which numerous strains or media were to be compared.

Temperature-gradient plate for growth in petri dishes. A satisfactory temperature gradient may be obtained in an apparatus which consists of two

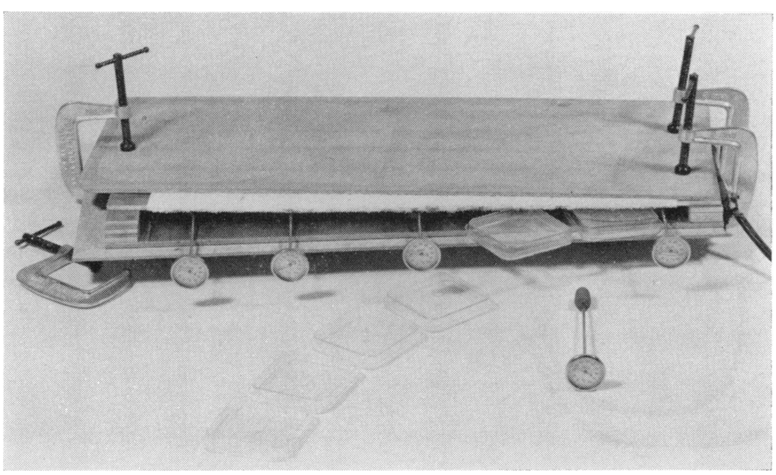

FIG. 2. *Aluminum sheet gradient plate, partly dismantled, showing strip heater (right), stem thermometers affixed with modeling clay, top and bottom Transite insulation, and square plastic petri dishes.*

0.6-cm aluminum sheets arbitrarily cut to 72.4 by 25.4 cm, petri dishes, and two sets of 25.4 by 3.8 cm aluminum strips aggregating to two 1.9-cm thicknesses. In this arrangement (Fig. 2), the aluminum sheets are spaced 1.9 cm apart by the aluminum strips on each end, and are covered with Transite sheets for insulation. The strip-heater unit (or an ordinary hot plate) is applied against one of the 25.4 by 3.1 cm edges so that both top and bottom sheets are heated equally. When temperature equilibrium has been reached, inverted petri dishes (preferably square, plastic petri dishes, which can form an almost continuous band of agar) are slipped between the aluminum sheets so that tops and bottoms of all dishes are in firm contact with the surfaces. If the petri plates are too uneven to make good contact with the aluminum, turkish toweling may be used between metal and plastic. Temperature readings at various points along the gradient can be obtained by inserting thermometers into balls of modeling clay and sticking these balls at appropriate points along the inside surface of the top or bottom aluminum sheet. That this procedure gives correct temperature readings is indicated by the smooth temperature curves obtained when thermometers inserted directly into the agar through holes in plastic plates were alternated with thermometers in modeling clay. During an observation period of 48 hr, temperatures varied within a range of ±0.3 C.

The aluminum-sheet arrangement has some important advantages: It is easy to construct and easy to use, since only petri dishes need be renewed and the gradient can be left undisturbed indefinitely. Direct contact between agar and aluminum is avoided. Inoculation, or transfer of membrane filters to the individual petri dishes, can be carried out very rapidly so that cooling during these operations is minimized. If the petri dishes are sealed with tape, drying of the agar is greatly reduced and plates can be incubated for 7 days or longer. This has proved to be useful in measurements of temperature optima in L-form experiments (Landman and Ginoza, 1961).

Temperature-gradient plate for growth in liquid media. On many occasions it is desirable to grow organisms at graded temperatures in liquid media. A temperature gradient along a 0.6-cm-thick aluminum sheet can be used to provide such a graded series. In the simplest arrangement, 50-ml Erlenmeyer flasks have been taped to the aluminum sheets at different points along the gradient. Recently, the convenient device shown in Fig. 3

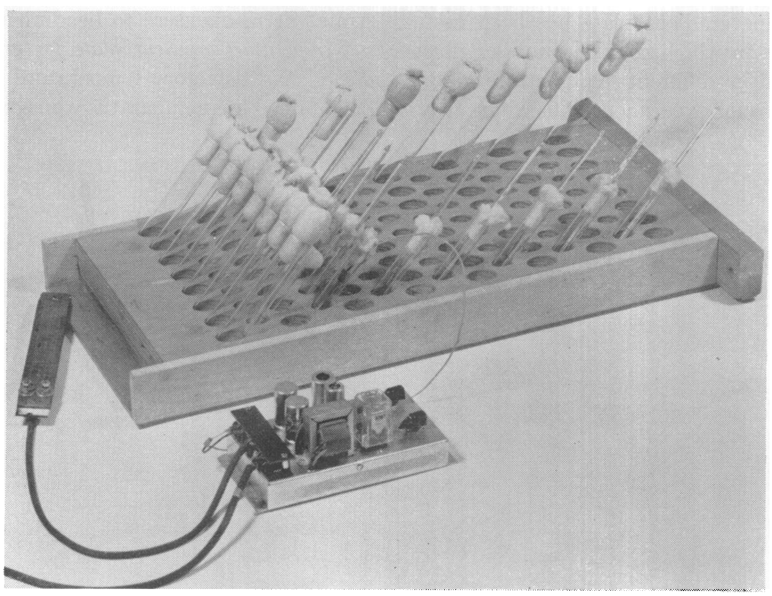

FIG. 3. *Aluminum-sheet gradient plate adapted for liquid growth. Only a narrow strip of the aluminum sheet is exposed on the left where the strip heater is placed. The sensing element of the thermistor is here arbitrarily inserted into a test tube. Alternatively, a flat sensing element may be applied directly to the strip heater.*

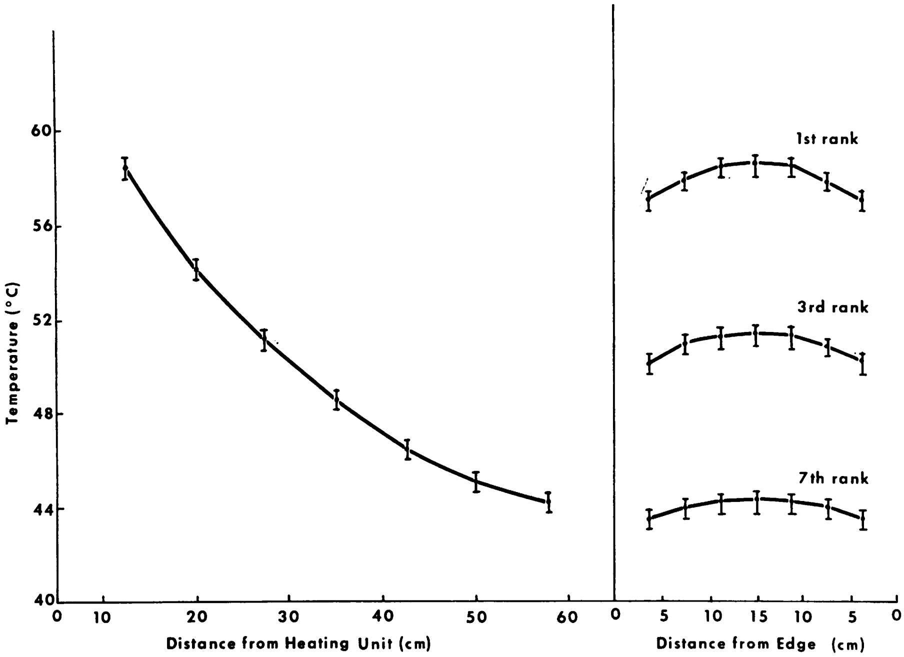

FIG. 4. *Temperature readings registered during a 36-hr period in water-containing test tubes positioned along and across an aluminum-sheet temperature gradient. Maximal and minimal temperatures for each tube are indicated by top and bottom of the I mark. The gradient plate was agitated on a reciprocating shaker in a 37-C constant-temperature room during the period of observation.*

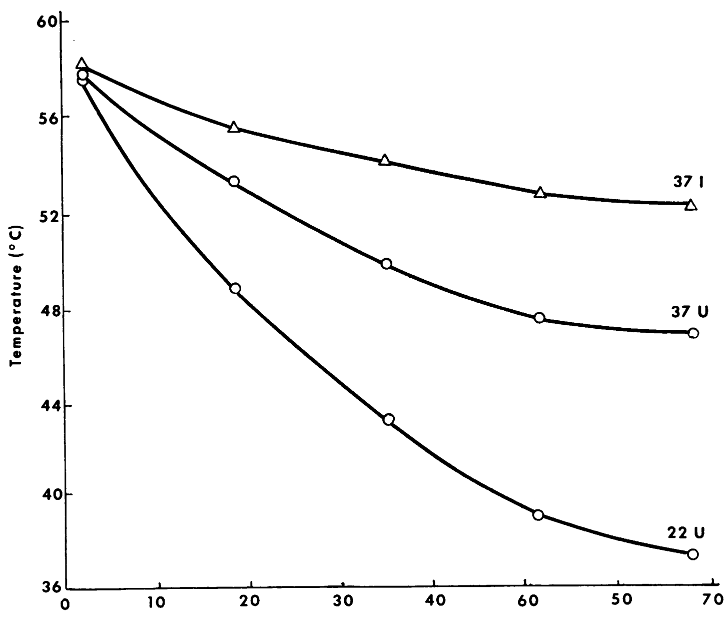

FIG. 5. *Temperature gradient variations obtained, with the wide-lane plate at a given maximal temperature setting, by changes in ambient temperature and insulation. Curve 37 I: gradient plate insulated with Transite sheets and toweling on top and bottom, incubated at 37 C ambient temperature. Curve 37 U: uninsulated plate at 37 C ambient temperature. Curve 22 U: uninsulated plate incubated at 22 C ambient temperature.*

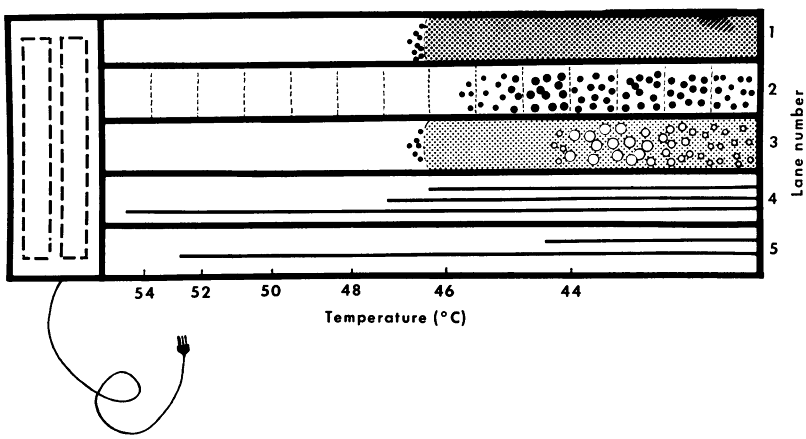

FIG. 6. *Various applications of the temperature-gradient plate. Lane 1 shows confluent growth of Bacillus megaterium on the nutrient agar below the sharp boundary line, isolated mutant colonies above it. Lane 2 shows that the colony count per unit area is constant right up to the boundary. The boundary is slightly below that of lane 1 because of the sparser inoculum (see Fig. 7). Lane 3: the temperate phage shown here displays a poorer temperature tolerance than its host strain; with increasing temperature it becomes increasingly virulent (clearer plaques) and its growth rate relative to that of the host also increases (larger plaques). Lane 4: streak 1 is the parent strain of B. megaterium shown in lanes 1, 2, and 3; streak 2 is a temperature mutant picked from an isolated colony in advance of the growth front; streak 3 is a thermophilic bacterium, B. licheniformis. Medium is nutrient agar. Lane 5: this lane shows the maximal temperature tolerated by the B. megaterium parent strain and the thermophilic strain of B. licheniformis on minimal medium.*

was put into use. It is a 5.7-cm-thick, 68.6 by 31.1 cm wooden block made of three layers of plywood. Through it, eight rows of eight 2.5-cm holes alternating with seven rows of seven 1.9-cm holes have been bored at a 45-degree angle. These holes accommodate, respectively, 25 by 200 mm and 18 by 150 mm test tubes. The block and the strip heater are fastened to the 0.6-cm aluminum sheet, covered on the bottom with a 75.6 by 34.3 by 0.6 cm sheet of Masonite. Wooden strips, 75.6 by 6.4 by 1.3 cm along the sides, provide additional insulation. The test tubes are inserted into the holes so that their bottom ends are in contact with the aluminum. The entire apparatus is clamped to a reciprocating shaker. Figure 4 shows the temperatures exhibited by water in the various test tubes at a given rheostat setting. In hourly readings, these temperatures stayed constant within ±0.5 C for 36 hr.

Factors affecting gradient characteristics. In all the foregoing gradient-plate arrangements, the characteristics of the gradient may be varied within wide limits. A high rheostat setting, used in conjunction with a light-gauge uninsulated aluminum plate in a cold environment, results in the steepest gradient. Figure 5 shows three gradients obtained with the wide-lane plate at different

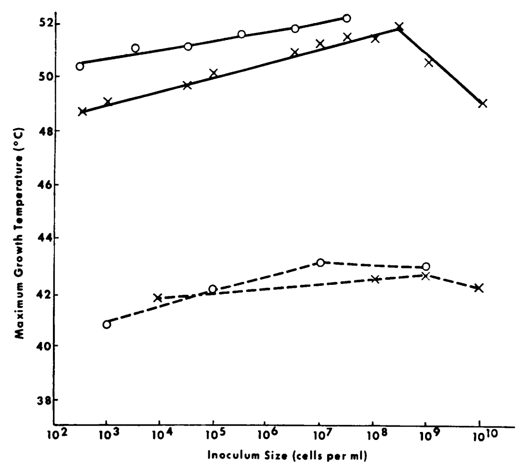

FIG. 7. *Effect of inoculum size and growth phase on maximal temperature of growth. Bacillus globigii on brain heart infusion agar: ○——○, log-phase inoculum; ×——×, stationary-phase inoculum. Escherichia coli B/r on minimal agar ○---○, log phase inoculum; ×-----×, stationary-phase inoculum.*

ambient temperatures, with and without insulation at the same maximal temperature setting. Gradients covering a variety of temperature ranges have been used, e.g., 37 to 56, 3 to 25, and 47 to 52 C. In a shallow gradient, such as the last-mentioned one, a temperature drop of 1 C may extend over a distance of 12 cm or more, so that very precise measurements of the effect of temperature on growth can be made.

APPLICATIONS

The temperature gradient plates have been used in a variety of experimental projects. Results from several of these will be described here briefly, since they illustrate the usefulness of the apparatus.

Determination of maximal growth temperatures of bacteria. When a 43 to 55 C gradient in nutrient agar on the wide-lane gradient plate is inoculated with a large number of cells of *Bacillus megaterium* strain C, a sharp boundary will appear at 46.3 C after 24 hr, with confluent growth below the boundary and, except for some isolated colonies near the boundary, no growth above it (Fig. 6). When analyzed with a dilute inoculum, all cells survive to give rise to colonies right up to the growth front, while none survives beyond it (Fig. 6). Just below the front, growth is slower, however, than at less extreme temperatures. By means of membrane-transfer experiments, it was shown that death occurs more and more rapidly as the distance from the boundary increases.

The exact location of the boundary depends upon a variety of factors. Figure 6 shows that the location of the growth fronts of *B. megaterium* may vary by as much as 2.3 C, depending on the growth medium. The inoculum size, as well as its growth phase, influences the maximal growth temperature (Fig. 7). The boundary first advances, and then recedes slightly with increasing inocula.

Isolation of mutants. One of our major objectives in developing the temperature-gradient plate was the isolation of mutant strains exhibiting increased temperature resistance. Despite extensive experimentation with untreated and mutagen-treated populations of 10^{10} or more bacteria of various species, we have not succeeded in recovering thermophilic mutants from mesophilic populations. It has proved possible, however, to isolate from several strains of bacteria mutants that show a *slightly* increased maximal growth temperature. These are the isolated colonies that appear in advance of the growth front illustrated in Fig. 6. In *B. globigii*, such gains were from 2 to 3 C. It should be pointed out that mutants of this type have been isolated and studied previously (Mefferd and Campbell, 1952).

Study of physiological effects of temperature. The utility of a continuous gradient for the study of a physiological effect of temperature is exemplified by the phage experiment illustrated in Fig. 6. From the experiment with *B. megaterium* strain C and phage M 5 (Friedman and Cowles, 1953), the following conclusions may be derived. (i) Phage M 5 (in contrast to other *B. megaterium* phages) ceases to multiply at a temperature below the maximum tolerated by its host. (ii) Up to its maximal growth temperature, phage M 5 maintains a fairly constant efficiency of plating through a wide temperature range. (iii) To judge from the relative turbidity of the plaques, lysogenization occurs more readily at the lower temperatures. (iv) To judge from plaque sizes, the "growth" rate of the phage relative to that of the bacteria is increased at the higher temperatures, and is much reduced at the temperature limit of the growth range of the phage.

ACKNOWLEDGMENT

The able and efficient cooperation of the Fort Detrick Metal and Woodworking Shops is gratefully acknowledged.

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