A TEMPERATURE-GRADIENT BAR AND ITS APPLICATIONS TO THE STUDY OF TEMPERATURE EFFECTS ON THE GROWTH OF REITER'S TREPONEME

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ABSTRACT

CANNEFAX, GEORGE R. (Communicable Disease Center, Atlanta, Ga.). A temperature-gradient bar and its applications to the study of temperature effects on the growth of Reiter's treponeme. J. Bacteriol. **83:**708–710. 1962.—A temperature-gradient device is described which employs a constant heat source and an aluminum bar with 294 holes to accommodate small test tubes. Examples of the range and degree of temperature gradients are presented, and the factors affecting heat conduction and heat losses are discussed. Three temperature gradients obtained under different conditions are shown graphically. The usefulness of the temperature-gradient bar in determining the optimal temperature for the cultivation of Reiter's treponeme is presented. The optimal temperature range was found to be 33 to 35 C. The potential usefulness of the temperature-gradient bar in other biological studies is suggested.

A complete study of growth response or thermal death rates of bacteria by the use of conventional incubator or water-bath temperature-controlling techniques is laborious and time-consuming. The temperature-gradient device described in this report provides a means for the maintenance of 49 temperatures for short or long periods of time.

The purpose of this paper is to present the details of construction of a temperature-gradient bar which employs small test tubes, together with temperature gradients obtained under certain conditions and the growth response of Reiter's treponeme between 28.0 and 39.4 C. The potential usefulness of the temperature-gradient bar in other biological studies of time and temperature is discussed.

APPARATUS

The temperature-gradient bar (Fig. 1) consists of an aluminum bar (2 in. by 2 in. by 4 ft) in which 294 holes (six rows with 49 holes in each row) are drilled. The holes are spaced ¾ in. apart (center to center); they are 1¾ in. deep and 5⁄16 in. in diam. The holes begin approximately 6 in. from the ends of the bar. These holes will accommodate ¼-dr flat-bottom shell vials which measure 7.5 by 50 mm. The bar is supported by two aluminum bars (1 in. by 4 in. by 13 in.). The supports are secured by three countersunk flathead metal screws 3 in. long (¼ by 20). The opposing surfaces of the bar and support are machined to provide a uniform metal-to-metal contact.

The bar is heated at one end by placing one support in an oil bath provided with a heater, thermoregulator, and constant stirrer. The bath will maintain the desired temperature within ±0.1 C. Oil was considered preferable to water, since oxidation and encrustation of the aluminum occurred with the latter. Also, the level of the oil in the bath can be maintained without the use of an automatic filling and level control, which is required when water is used. After the oil has reached the desired operating temperature, the level is adjusted to a mark 9 in. from the bottom of the support.

Observations on the growth of Reiter's treponeme were made by observing changes in optical density with time. The medium was Spirolate broth (BBL) supplemented with 10% inactivated normal rabbit serum. Gelperin (1949) has shown that there is a linear relationship between the concentration of these treponemes and optical density measurements; unpublished data of this

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laboratory have corroborated his observations. A spectrophotometer with a cuvette holder for use with tubes of approximately ¼-in. diam has been used for determining optical densities at 550 mμ. The use of paraffin-coated cork stoppers permits mixing of the tubes by inversion. A tab of cellophane tape attached to the stoppers facilities insertion and removal of the tubes from the cuvette holder.

Temperature measurements of fluid in the tubes were made with a thermistor-type thermometer which is sensitive to small changes in temperature and has a rapid response. The thermistor probe may be placed in a tube and the instrument connected to a recorder, to give a permanent record of the constancy of the temperature of any given tube throughout an experiment. Ambient temperature measurements were made with a paper-disc recording thermometer.

DETERMINATION OF TEMPERATURE GRADIENTS

Examples of temperature gradients are shown in Fig. 2. In curve A, the oil-bath temperature was 60 ± 0.1 C; in curve B, the temperature was 80 ± 0.1 C. The ambient temperature with constant air circulation in each instance was 25 ± 1 C. The oil-bath level was 9 in. It can be seen that the temperature values for the 49 rows of tubes, plotted on semilogarithmic paper, result in a shallow temperature gradient. The temperature increment between adjacent tubes is not uniform, since the temperature gradient becomes more steep as the heat source is approached. If one should desire a shallow gradient within a certain range, the conditions which affect radiant heat loss or heat conduction may be adjusted to provide the desired gradient. For example, by changing the oil-bath temperature from 60 to 80 C, the temperature at tube 17 (with 60 C heat input) and tube 49 (with 80 C heat input) is the same (32.6 C), but the increment between tubes 17 and 16 is 0.3 C and the increment between 49 and 48 is 0.2 C. Thus, the temperature gradient at the lower end of that provided by 80 C heat input is less than the gradient near the mid-range of the gradient provided by 60 C heat input in the range of 32.6 C.

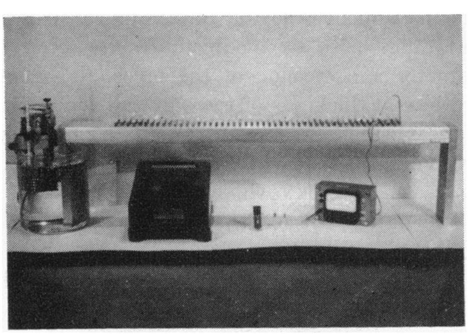

FIG. 1. *Temperature-gradient bar and accessories.*

It is possible to obtain almost any gradient required for biological studies by control of heat input and heat loss. Heat loss may be altered by insulation of the bar, changing the ambient temperature, and control of air circulation near the bar. Still different gradients may be obtained by immersing the second bar support in a bath that is either lower or higher than ambient temperature while holding the other heat-loss factors constant. Curve C of Fig. 2 is an example of the use of two baths. The conditions producing curves B and C were the same except that curve C

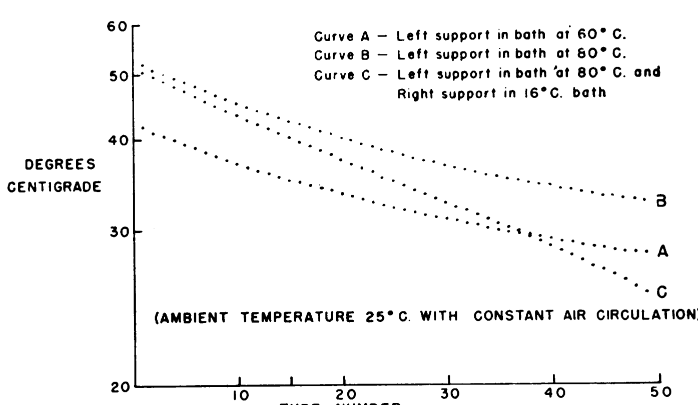

FIG. 2. *Examples of temperature gradients.*

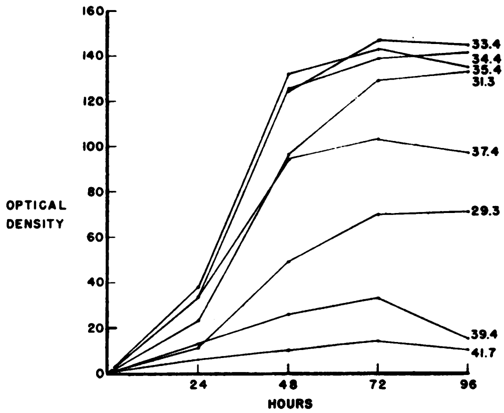

FIG. 3. *Growth curves of Reiter's treponeme at selected temperatures.*

was obtained by immersing the other support in a bath at 16 C. It is obvious from these three examples that an infinite number of gradients may be possible by altering heat conduction and heat-loss factors.

PRACTICAL APPLICATION OF THE TEMPERATURE-GRADIENT BAR

The growth responses of Reiter's treponeme at approximately 1 and 2 C intervals between 29.3 and 41.7 C are shown in Fig. 3. The optical-density values are the average values of three experiments. In each experiment, there were five growth tubes at each temperature. There were 49 growth responses in all, but for clarity of presentation, only 8 of the 49 are shown in Fig. 3. It is apparent that the greatest early and total growth responses were observed in the range of 33.4 to 35.4 C. It is also apparent that 37.4 C had a suppressive effect on growth. The generallv recommended temperature for the cultivation of Reiter's treponeme has been 37 C. The present data demonstrate that the optimal growth temperature lies between 33 and 35 C. It was also found that the optical density of the growth medium alone increased with time. The data in Fig. 3 were corrected by the control medium values.

DISCUSSION

There are two temperature-gradient devices reported in the literature, each of which apparently served the purpose for which it was intended. The temperature-gradient bar described in this paper possesses greater flexibility and general usefulness than the two devices previously described. The two previously described temperature-gradient devices, employing an agar sheet (Halldal and French, 1958) or a trough of agar (Bausom, Landman, and Matney, 1960) seeded with organisms, are restricted for the most part to investigations of the effect of temperature on the growth of aerobic organisms. The temperature gradient device reported here may also be used for the study of: anaerobic organisms, thermal death rates, nutritional requirements, effects of antibiotics, storage decay of biologicals, and optimal temperatures for serological reactions.

It is obvious from the above that the temperature-gradient bar in its present form may be employed in the investigation of time and temperature relationships in many biological studies. The instrument is flexible in that its construction and range may be modified, by changing the diameter and spacing of the holes, the length, width, and thickness of the bar, and the amount of heat supplied, to suit particular problems involving time and variable temperatures.

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LITERATURE CITED

BAUSOM, H. T., O. E. LANDMAN, AND T. S. MATNEY. 1960. The temperature gradient plate and its applications. Bacteriol. Proc., p. 55.

GELPERIN, A. 1949. Morphology, cultural characteristics and a method for mass cultivation of the Reiter spirochete. Am. J. Syphilis Gonorrhea Venereal Diseases **33**:101–113.

HALLDAL, P., AND C. S. FRENCH. 1958. Algal growth in crossed gradients of light intensity and temperature. Plant Physiol. **33**:249–252.