sank the concentration of Pyruvat and Acetaldehyde. Man kann daraus folgern, dass das Acetyl-methylcarbinol bei diesen Actinomyceten aus Pyruvat und Acetaldehyde synthetisiert wird, ähnlich wie wir bei S. erythreus (auch aus freiem Acetaldehyde allein) in vitro festgestellt haben.


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Summary

The production of acetyl-methylcarbinol by various strains of actinomycetes was established. The biosynthesis of acetyl-methylcarbinol depends largely on the composition of the fermentation medium and on the presence of arsenite, which regulates the metabolism of pyruvic acid. In the case of Streptomyces aureofaciens the stimulating effect of arsenic on the production of acetyl-methylcarbinol gives a partial explanation of the well-known negative effect of phosphate on the biosynthesis of chlortetracycline.

A Study of the Temperature Dependence of Radiation Sensitivity of Dry Spores of Bacillus Megaterium Between 5° K and 309° K

The temperature dependence of effects of X-rays on biological systems has been the subject of several recent investigations; however, none of those studies included temperatures below 77° K. In view of the theoretical importance of the temperature dependence relationship, the temperature range was extended in this study to 5° K to provide a complete description of it. A brief report has appeared previously.

Spores from two nonlysogenic strains of Bacillus megaterium (No. 337, Welshimer and ATCC No. 8245) were mounted in known concentrations on membrane filters and dried in vacuo according to the method of Powers et al. The X-ray source was a Machlett OEG 60 tube, with a tungsten target and a beryllium window, operated at 50 kV and 45 ma without added filtration (HVIL 0.070 mm Al). The irradiation chamber was a gas tight stainless steel cylinder, 5 cm by 41 cm, attached directly to the X-ray tube (Fig. 1). The membranes carrying the spores on their surfaces were irradiated in stacks of five at a target distance of 45 cm at a dose rate of 2800 r/min (measured in air with a windowless ionization chamber) to the first filter (2400 r/min to the fifth filter). Anaerobic conditions were achieved in the exposure chamber by evacuating to 1 mm Hg pressure and flushing with purified helium twice before sealing the chamber at one atmosphere. The temperature of the spores was fixed by immersing the exposure chamber in appropriate liquid and slush baths. The double Dewar apparatus illustrated in Figure 1 was designed to meet the requirements of liquid helium, but was applicable to the other baths. The temperature of the membrane filters was monitored during all irradiations with two thermocouples in contact with the spore-bearing filters. The temperatures at which experimental points were obtained were observed to be as follows: 309° K (water bath); 299° K (water bath); 274° K (ice water); 253° K (NaCl-saturated ice water slush); 196° K (dry ice-acetone); 152° K (95% ethanol slush); 79° K (liquid nitrogen); 64° K (nitrogen slush); 21° K (liquid hydrogen); 6° K (liquid helium); and 5° K (liquid helium).

Fig. 1.—Diagram of the apparatus for controlling temperature during irradiation.
The membrane filters were returned rapidly to about 25°C following the completion of the X-ray exposure and were maintained at this temperature until plated 1–8 h later on a buffered peptone glucose medium (double strength Difco M.R.-V.P.). Colony counts were made after 16–18 h incubation at 35°C.

The biological effect measured was loss by the irradiated spores of colony-forming capacity. Using direct spore counts as the comparative measure, we ascertained that control (zero dose) preparations of spores demonstrated statistical 100% colony-forming capacity (= survival) at all temperatures. This observation of survival of the spores at the very low temperatures at prolonged times is of interest in itself, and is similar to the one other observation of this kind known to us in which spores of bacteria and molds were exposed in vacuo to the temperatures of liquid hydrogen and liquid helium without loss of germinating power.

The dose-survival curves at all temperatures are sigmoid with a small shoulder at high survival values. Of a number of possible ways of describing them, we have chosen

$$N/N_0 = e^{-kD}$$

(1)

in which $D$ is radiation dosage and $k$ is the slope of the exponential portion of the inactivation response curve. In this case $n$ is the $y$-axis intercept of the extrapolated exponential curve. Use of this function, which is convenient because of the ease of estimating values of $k$ and $n$, yields values of $k$ and $n$ very close to those given by the preferred and more complete description

$$N/N_0 = 1 - (1 - e^{-kD})^n.$$  

(2)

In these experiments no survival values above 50% were used in evaluating $k$ and $n$ and, since the shoulder exists only at values higher than these, the constants in equation (1) are very close to those of equation (2).

The temperature at which the two sets of points appear to intersect is about 130°C. However, no measurements were made in this region, and a sharp change from a temperature independent condition to a temperature dependent condition is not required by the data—the change may occur gradually over a range of temperatures.

The other constant in the equation used in the numerical analysis of these results is invariant with temperature. Figure 3 presents the value of $n$ of equation (1) as related to temperature, our interpretation of the relationship being that $n$ is varying randomly about a mean value of 1.30 as the temperature at the time of irradiation changed.

These experiments show for the first time in a biological system the existence of a transition region in the temperature dependency of radiation inactivation at about 130°C; this is characterized by temperature dependence above the transition region, and temperature independence below it. It is probably significant that these relationships are consistent in two respects with observations made previously in another biological system in this laboratory by Bachofen et al. First, the relationship of the X-ray inactivation slopes to temperature for the dry bacteriophage T1 is such that there may be a temperature-dependent region and a temperature-independent region with the transition occurring at approximately 130°C. Second, recalibration of the activation energy for the dry virus (assuming the 130°C transition point) yields a value of about 200 cal, a value in the order of the 110 cal reported by Houtermans.

The phenomena, then, probably occur in more than one kind of spore, and the

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7 T. Houtermans, Z. Naturf. 9b, 600 (1954); 11b, 636 (1956).