

BACTERIOLOGY¹

Chairman: EDITH HAYNES, Indiana University

R. C. Bard, Indiana University, was elected chairman for 1952

ABSTRACTS

Temperature effect on radiation damage in bacteriophage. C. S. BACHOFER, University of Notre Dame.—In order to establish the effect of temperature on the *direct* action of ionizing radiation in biological systems, bacteriophage T1, specific for *Escherichia coli*, strain B, was irradiated with x-rays at temperatures from 77° K to 310° K. Material was irradiated *in vacuo* in a specially constructed chamber to prevent frosting, liquefaction of air, and interference of oxygen. Three series of experiments utilized 1) phage in broth, lyophilized; 2) phage in broth, vacuum-dried; 3) phage in chemically defined medium, vacuum dried.

No inactivation due to temperature alone was observed at temperatures as low as 77° K or as high as 310° K, for the duration of the experiments.

Experimental results show that survival of bacteriophage after irradiation with a given dose of x-rays is inversely proportional to the temperature at which they are irradiated, that is, there exists an inverse correlation between radiation damage and temperature. The relationship between temperature-of-irradiation and survival varies with the physical state of the irradiated virus. This has been defined mathematically for the three sets of experiments listed above, as follows:

1. Phage in broth, lyophilized.

The relationship between titer and temperature is

$$y = a - 1.54x$$

where y is the titer, expressed in millions of phage particles per ml, at a given temperature-of-irradiation x , when a is the titer at 0° K.

2. Phage in broth, vacuum-dried.

$$y = a - 3.28x$$

3. Phage in chemically defined medium, vacuum-dried.

$$y = a - 1.65x$$

Respiration of *Clostridium perfringens*. R. C. BARD, Indiana University.—The obligate anaerobe, *Clostridium perfringens*, utilizes oxygen at a rapid rate in the presence of glucose. The respiratory rates are comparable to those of aerobic organisms with $Q_{O_2} = 110-180$, $Q_{CO_2} = 190-310$, $RQ = 1.6-2.0$. Respiration is not inhibited by 0.001

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M potassium cyanide suggesting the absence of heme-type respiratory catalysts. A flavoprotein-type respiration may be operative but hydrogen peroxide does not accumulate nor does addition of large amounts of catalase reduce the quantity of oxygen consumed. Inorganic phosphorus stimulates the rate of respiration and is converted to high energy phosphate compound(s) although stable phosphate compound(s) also appear to be formed. Iodoacetate and α, α' -dipyridyl inhibit respiration. The former finding suggests the participation of sulfhydryl enzyme(s) involved in respiration. The latter finding indicates the probable functioning of the metallo-aldolase known to be present in this organism (Bard and Gunsalus, 1950). Iron-deficient cells respire weakly with the accumulation of pyruvate. This finding suggests a role of iron in respiration, probably indirect in the sense that the pyruvate dissimilatory mechanism is iron-dependent, either for its formation during growth or for its operation during glucose and pyruvate breakdown. The data thus far do not permit a clear statement describing this respiratory mechanism. The presence of such a mechanism is an obligately anaerobic organism, incapable of growth in the presence of oxygen, poses an interesting problem.

Some considerations in the use of the soil perfusion apparatus for microbiological studies. LLOYD R. FREDERICK, Purdue University.—An adaption of the Audus modification of the soil perfusion technique used by Lees and Quastel is described in detail.

Some advantages of the technique are as follows: the soluble and gaseous products of transformations by a heterogenous population may be determined without disturbing the soil sample; the gas used may be controlled; there is very little change in moisture content; additions to the system can be made readily.

Some of the disadvantages are as follows: it is very difficult to use soils in which there are no stable particles larger than 0.25 mm. diameter; studies can be made only under nearly saturated conditions; channels develop which may cause areas that are not in equilibrium; some microbial growth may take place in the perfusate. These difficulties are minimized when 1—5 mm. table aggregates are used and the rate of perfusing is fairly rapid with a high ratio of air to water.

This technique is valuable in the study of various factors on the release of soluble materials and the transformation of compounds by microorganisms. Pure cultures can be obtained from the enriched soil. In our studies the oxidation of sulfur caused more manganese to become soluble and the resulting low pH prevented a reversion to the insoluble form.

A comparison of three media used at the Indiana State Board of Health in culturing for tubercle bacilli. C. F. HILL and S. R. DAMON, Indiana State Board of Health.—Two thousand diagnostic specimens, including sputum, gastric washings, urine, and spinal fluid, were cultured for tubercle bacilli in the laboratory of the Indiana State Board of Health. The media used were Jensen's modification of Lowenstein's

formula, Frobisher's modification of Petraghani's formula, and Peizer's Medium.

Special attention was given to the following: 1. Which medium yielded the largest number of recoveries; 2. Which medium gave earliest growth; 3. What combinations of media appear most profitable from the standpoint of total recoveries; 4. Which medium is least satisfactory because of overgrowth by contaminants; 5. Productivity with relation to the length of incubation period.

The results indicated that: 1. Lowenstein's medium was the most satisfactory; 2. Lowenstein's medium gave earliest growth; 3. Lowenstein's and Peizer's media made the best combination; 4. Overgrowth by contamination was greatest on Petraghani's medium; 5. Cultures should be observed at least six weeks.

Use of new microtechniques for bacterial identification. L. S. MCCLUNG and E. D. WEINBERG, Indiana University.—A new type of micro method for the rapid identification of biochemical abilities of bacteria is described. The abilities include fermentation of carbohydrates; decomposition of amino acids; hydrolysis of proteins, polysaccharides and lipids; and reduction of inorganic salts.

Whereas previously described rapid methods allow quick multiple action of the culture, this method involves the addition of large, washed inocula to preheated tubes or plates containing substrate and indicator but no other nutritives. The omission of nutritives prevents extensive cell multiplication; thus the visible indicator changes presumably result from the activities of cells contained in the inoculum rather than from variants that arise and further multiply in the presence of the substrate.

In carbohydrate fermentation tests of this type, rapid indicator changes (within 15-30 minutes) reflect the activities of constitutive enzymes; slow indicator changes (2-4 hours) reflect the activities of adaptive enzymes. Prior growth of the cells in the presence of the substrate converts slow indicator changes to rapid changes.

On the chemical nature of the Rh factor. MERWIN MOSKOWITZ, Purdue University.—The stability of the Rh factor in human red cell stroma has been tested after being treated with the following agents: trypsin, guanidine, papain, hyaluronidase, *Clostridium welchii* Type A toxin, ultraviolet irradiation, and pyridine. All these agents, with the exception of hyaluronidase, destroyed the Rh factor. These experiments lend support to the hypothesis that the Rh factor is of protein nature, or else is intimately associated with a protein component in the red cell.

Preliminary studies on a cell-free amylase of *Bacillus stearothermophilus* ATCC 7954. EGON STARK and P. A. TETRAULT, Purdue University.—The isolation of bacterial, cell-free, starch saccharifying enzymes from the medium at 70°C is described (J. B. 62: 247, 1951). A crude enzyme preparation obtained and tested at 65°C for 4 hours saccharified 1 per

cent soluble starch strongly, 0.5 per cent dextrin weakly and 0.5 per cent maltose not at all at pH 5.5 and 8.5 respectively. The following data are preliminary and indicative rather than characteristic. Saccharification proceeded slowly over a wide range of temperature (25°C-95°C) and pH (2.5-11). Optimum temperature 65-75°C; optimum pH 5-7. Better activities above 65°C and below pH 7. Glucosidases acting strongly at very alkaline pH cannot be excluded. Limits of conversion for cell-free enzyme 52-58 per cent, in presence of cells 55-77 per cent. Sixty per cent arbitrarily selected for calculations. Universal buffer (borate, acetate, monosodium phosphate) better than Sorensen's phosphate buffer. Calcium carbonate depressed saccharification of the fermentation medium. Methods and their shortcomings are discussed. Results are shown on slides.

The effect of subfreezing temperatures on pathogens. R. W. SQUIRES and S. E. HARTSELL, Purdue University.—Cell suspensions of *Micrococcus pyogenes* var. *aureus*, *Escherichia coli*, #7006, and *Salmonella aertrycke* were quick frozen at the stage of physiological youth and stored at -9°C. from one to fourteen days. Growth curves and characterizing biochemical reactions were determined before freezing and immediately after defrosting of each organism. Fermentation studies and other differential tests were determined by using microtechniques. Growth curves were plotted from turbidimetric data.

Results indicated that, in a few instances, subfreezing temperatures contribute to the acquisition of certain biochemical characteristics. After defrosting, *M. aureus* was observed to ferment xylose, raffinose (with acid, but no gas) and to give a positive test for acetyl methyl carbinol. These changes, observed consistently, do not remain stable in succeeding generations. No significant variations were observed for *E. coli* and *S. aertrycke*.

Turbidimetric data for defrosted cells indicated that subfreezing temperatures may contribute to some deviations in the growth curve and to an increase in the lag phase. The growth curve for *S. aertrycke* was observed to depart noticeably from that of the control as the time of storage was lengthened. The forms of the growth curve for *M. aureus* and *E. coli* were not altered at the storage times studied.

The lag phase of all species appeared to be a function of the storage period. As the time of storage increased, the longer the refrosted cells remained in the lag phase. The time required for refrosted cells to initiate growth (the recovery period) seemed to be independent of the size of the inoculum employed. Light and dense suspensions of defrosted cells required similar lag times before the initiation of growth.