pump which mixes the buffer solutions surrounding the two electrodes, thereby neutralizing changes in the *p*H or concentration of the buffer as a result of electrode reactions (*4*). The heat developed in the cell (*5*) by passage of the electric current is removed by cooling plates in the walls of the buffer chamber. The two electrodes are made of stainless steel, and their large area (10 × 10 cm) provides a reasonably uniform electric field through the face of the gel slab. The electrodes are placed in close proximity to the gel matrix and to the separating grid in order to decrease the heat load generated within the apparatus by the passage of the electric current. A relatively low voltage and high current are required (25 v at 200 to 500 ma) (Fig. 1).

There are certain necessary conditions and limitations for the apparatus to function effectively. First, the proteins must all migrate in the same direction through the gel and through the buffer solution. This, in effect, precludes the use of starch gel as electrophoresis matrix since some slow moving components, such as γ-globulin, which migrate toward the negative electrode in the starch gel matrix, migrate toward the positive electrode in the buffer solution. In such a case, the protein could never set up within the vertical channel the electroconvection currents necessary to concentrate the protein in the collecting reservoir at the bottom. Presumably a cell could be designed with electroconvection channels on both faces of the gel matrix to accommodate both forward and retrograde migrating proteins, but the additional complications in the design of such an elution cell seem to be greater than the useful applications such a cell would warrant.

Second, the individual proteins comprising the eluting zones must have a positive density increment in the buffer used. In other words, the protein-buffer solution must have a higher density than the protein-free buffer. If this condition is not met the eluted protein will tend to float to the top of the cell instead of descending by convection to the bottom. Again, it might be possible to provide collecting reservoirs at the top of the elution cell to meet this difficulty, although the area of usefulness of such a design would be extremely limited.

Finally, an important requirement is that the protein must be large enough to be nondialyzable, that is, it must not pass through the dialysis membrane used to form the base of the electroconvection channel. The usual Visking dialysis tubing effectively bars the passage of proteins whose molecular weight is 20,000 or more, so that this is not a serious limitation in the applications of the cell.

An example illustrating the use of this apparatus is presented in Fig. 2. The sample consisted of an artificial mixture of five colored proteins and three dialyzable dyes. A section of the gel pattern before elution is shown placed on the separating grid in position for elution. The protein components, collected from another section of the original gel pattern, are visible in the collecting tubules, while the dye components, having dialyzed through the barrier membrane, were not concentrated in the tubules corresponding to these bands. The original photograph clearly shows in two colors the separation of the two components on the left (closest to the origin) in the gel pattern.

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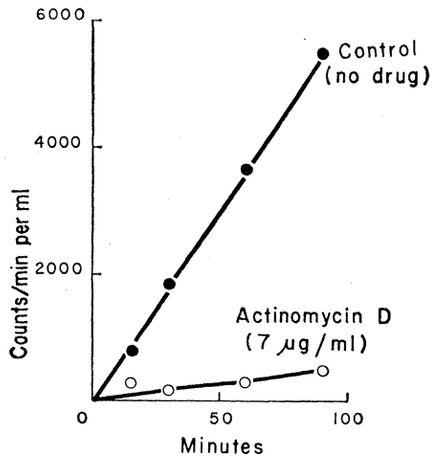
19 August 1964

Actinomycin Resistance in Bacillus subtilis

Abstract. *The incorporation of uracil-C^{14}, a precursor of cellular RNA, into whole cells of the sensitive parent strain* Bacillus subtilis *No. 6051 is completely inhibited by actinomycin D, whereas only partial inhibition of uracil-C^{14} incorporation occurs with actinomycin-resistant intact cells of* B. subtilis *strain II 15. Incorporation of uracil-C^{14} into protoplasts of the resistant strain of* B. subtilis *is completely inhibited. The sensitivity of resistant-cell protoplasts to drug effect clearly supports the conclusion that actinomycin penetration is altered in resistant intact whole cells. Since other experiments have shown that the antibiotic is not broken down in the presence of resistant cells, resistance is considered to result from a change in cell permeability.*

Actinomycin D has proved a useful tool in blocking RNA synthesis in many mammalian and microbial cell systems (*1*). It binds reversibly with the guanine moiety of DNA, the result being inhibition of DNA-dependent RNA-polymerase (*2*).

Little is known concerning the chemical and biological reactions occurring in the emergence of resistance to actinomycin D in any biological system susceptible to this antibiotic. Goldstein *et al.* (*3*) and Journey and Goldstein (*4*) characterized a HeLa line resist-



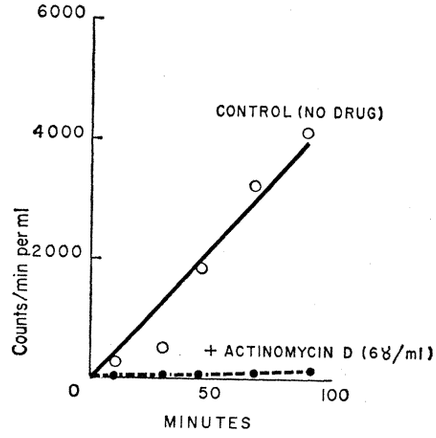


Fig. 1 (left). Uracil-2-C^{14} incorporation into *B. subtilis* strain 6015/S in the presence and absence of actinomycin D. Fig. 2 (right). Uracil-2-C^{14} incorporation into protoplasts of *B. subtilis* strain 6051/S in the presence and absence of actinomycin D.

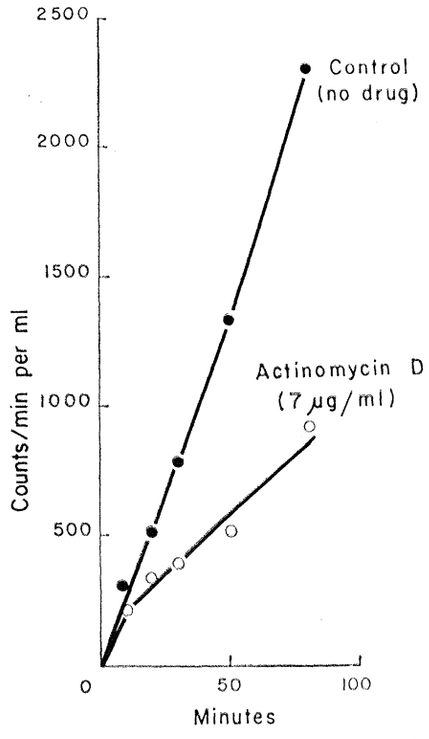


Fig. 3. Uracil-2-C^{14} incorporation into *B. subtilis* strain II 15/A in the presence and absence of actinomycin D.

ance to actinomycin in which development of resistance was assumed to result from (i) cell surface changes prohibiting entry of drug, (ii) drug binding or detoxification within cell, or (iii) enzyme inactivation of the drug. In microbial systems, the natural resistance of *Escherichia coli* to actinomycin D has been shown, by Haywood and Sinsheimer (*5*) and Mach and Tatum (*6*) who used protoplast suspensions, to be due to impermeability of intact cells to actinomycin. Our study was undertaken to establish a mechanism for the emergence of drug-resistant bacterial cell lines from sensitive parent strains.

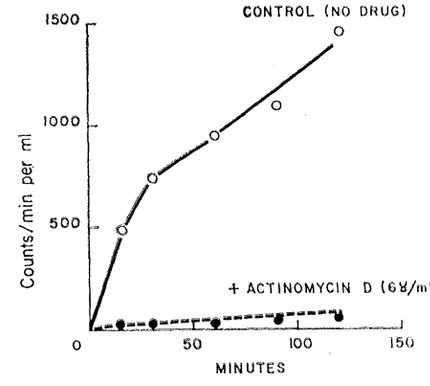


Fig. 4. Uracil-2-C^{14} incorporation into protoplasts of *B. subtilis* protoplasts II 15/A in the presence and absence of actinomycin D.

Strains of *Bacillus subtilis* (No. 6051 from the American Type Culture Collection) resistant to actinomycin D were isolated by the gradient-plate technique (*7*). Stock slants of drug-resistant isolates were maintained on heart-infusion medium that contained 100 and 1000 times as much actinomycin (10 and 100 μg per milliliter, respectively) as could be tolerated by a sensitive parent. Protoplasts were prepared with lysozyme (0.5 mg/ml) and 0.2*M* sucrose in *M*/15 phosphate buffer at *p*H 7.0. After 10 minutes incubation at room temperature, protoplasts were centrifuged, washed with the buffered sucrose solution, and suspended in Demain's synthetic basal medium (*8*) containing 1 percent glucose and 0.5*M* sucrose. Protoplast preparations were made from suspensions of intact cells adjusted to an optical density of 1.50 at 575 mμ. For studies with whole cells an optical density of 0.20 was used.

Sensitivity of either whole intact cells or protoplasts to actinomycin D was determined by measuring the incorporation of uracil-2-C^{14} into material insoluble in cold 5 percent trichloroacetic acid. Uracil-2-C^{14} (0.1 μc/ml and 10 μg/ml) was added to a suspension of protoplasts or whole cells incubated in the presence of actinomycin D (6 to 7 μg/ml) or its absence. Samples were removed at intervals and added to an equal volume of 10 percent trichloroacetic acid, filtered, and washed on Millipore filters as previously described (*9*). The radioactivity of the acid insoluble material was measured on a gas-flow counter.

The incorporation of uracil-C^{14}, a precursor of cellular RNA, into whole cells and protoplasts of the sensitive parent strain *B. subtilis* No. 6051 is completely inhibited by actinomycin D (Figs. 1 and 2) whereas only partial inhibition of uracil-C^{14} incorporation occurs with actinomycin-resistant intact cells of *B. subtilis* strain II 15 (Fig. 3). In contrast with the effect of actinomycin D on uptake of uracil-C^{14} into resistant whole cells, incorporation into protoplasts of the resistant strain of *B. subtilis* is completely inhibited (Fig. 4). The sensitivity of resistant-cell protoplasts to drug effect clearly supports the conclusion that actinomycin penetration does not occur in resistant intact whole cells.

Since other experiments have shown that the antibiotic is not broken down in the presence of resistant cells, resistance is considered to result from a change in cell permeability.

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Morphogenetic Studies with Partially Synchronized Cultures of Carrot Embryos

Abstract. *Callus tissue, derived from petiole segments of the wild carrot and containing undifferentiated meristems, can be suspended in liquid nutrient media and passed through a series of sieves to separate the meristems by size. The small meristems of fairly uniform size thus obtained will grow on defined media where their controlled differentiation into embryos can be studied.*

A simple method has been devised for studying morphogenetic problems through the use of partially synchronized cultures of embryos derived from somatic cells of the wild carrot, *Daucus carota*. Details of the embryogenesis which occurs in callus tissue derived from petioles, roots, or stems cultured on simple defined media were described elsewhere (*1*).

Segments of carrot petiole (*2*) cultured on an agar-solidified basal medium (*3*) plus adenine and 2,4-dichlorophenoxyacetic acid (2,4-D) produce callus which contains many small, approximately spherical, meristems. Such callus will multiply its fresh weight five- to tenfold during successive 30-day periods in subculture. The callus forms new meristems for a variable