grams displayed at lower magnification were made. The appearance of the spindle has been described by Kane (*6*) and, for MA *in situ*, by Harris (*7*) and Gross *et al.* (*5*). The fibers, in the spindle region, run in bundles or tracts which are separated by space of a few thousand angstroms to several microns. In the spaces there are very large numbers of small particles and some vesicles. The particles resemble ribosomes and may contribute a major fraction of the total protein of the isolates.

The field of the low-power autoradiogram (Fig. 4) contains the metaphase chromosomes and a number of fiber tracts, the latter clearly distinguishable by their increased electron density with respect to the nonfibrous matrix. This field is representative in that the silver grains tend to lie either directly over or very close to the fiber tracts. The field shown contains 26 silver grains (or perhaps a few more if some of those shown are double). Seventeen of these, at least, are directly over fibers or fiber tracts. This means that about 62 percent of the radioactive disintegrations that occurred during the period of exposure had their sites in or immediately adjacent to fibers which occupy a very small fraction of the total area of the specimen. Figure 5 shows, at higher magnification, the association of a silver grain with a pair of fine fibers and, in addition, the appearance of the nonfibrous matrix in the vicinity.

The electron microscope autoradiograms therefore confirm the impression given by the optical autoradiograms shown in Fig. 1. The radioactivity in the isolated MA tends toward a distinct association with the fibers, which represent the structural skeleton of the organelle. It might be argued that the fibers present a surface for nonspecific adsorption of radioactivity from labeled proteins in the matrices. But in that case one would expect to find only some label on the fibers, and not a strong tendency for it to localize on them. With regard to low molecular weight precursors trapped in the preparations, the routine fixation and embedding treatments render considerably more of the label soluble (and hence remove it) than does treatment with the usual protein precipitants, such as TCA. A similar conclusion has been reached by Caro and Palade (*18*) for other specimens.

Taken together, these results support the original interpretation of the acquisition of radioactivity by the MA during exposure to labeled amino acids, namely that proteins associated with the structure or function of this organelle are synthesized during cleavage in the sea urchin egg, as they must be in tissue cells (*19*), and that these proteins are an important fraction of the total made during early cleavage. They also justify an enlarged effort to separate and purify the proteins of the isolated MA by various methods, so that an accurate identification of the preexisting and newly synthesized species can be made (*20*).

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Agrobacterium tumefaciens: Thermal Inactivation of Tumor-Inducing Ability

Abstract. *Treating cultures of* Agrobacterium tumefaciens *at 39° to 48°C reduces the infectivity of the bacteria without necessarily affecting viability. Destruction of the capacity to initiate tumor growth follows first-order kinetics from which rate constants for thermal inactivation are derived. From these rates, values for heat of activation of 56.7 kcal mole^{-1} and entropy of activation of 107 cal mole^{-1} deg^{-1} are obtained. A particular protein or nucleoprotein active in the process of infection may be inactivated by the treatment.*

Treatment of plants infected with *Agrobacterium tumefaciens* (Smith and Town.) Conn, the organism that induces crown-gall tumors, at temperatures of 32° to 35°C inhibits tumor formation (*1, 2*). Braun (*3*) has calculated an activation energy for this process of 80 kcal mole^{-1} or greater. We used a quantitative assay, similar to the ones for plant viruses, for the infectivity of *A. tumefaciens* (*4*) to reexamine the effect of temperature treatments on the virulence of the bacterium without the complications involved in subjecting both bacterium and host to the treatments.

Shake cultures of *A. tumefaciens* strain B6 were grown for 48 hours at 27° ± 1°C in a medium consisting of Bacto nutrient broth and 0.1 percent yeast extract (*5*). Portions (50 ml) of such cultures in 250-ml erlenmeyer flasks were heated for various periods in a constant-temperature water bath. Temperatures ranged between 39° and 48°C. Samples (2 to 3 ml) were removed at various times and pipetted into sterile tubes held in crushed ice. Portions of each sample were then diluted for assaying infectivity and for making counts of viable cells. At least three different cultures were treated at each temperature. The infectivity of each sample was measured on 12 to 18 primary pinto bean leaves (*4*). Plate counts of viable cells were made from one or two dilutions of a serial tenfold dilution series; three plates were used to measure each dilution. The inoculation of both leaves and plates was usually

completed 7 to 8 minutes after the sample was removed from the water bath.

Only small variations in the viable cell count (Fig. 1) occur during the first 30 minutes at 39°, 42°, and 45°C, and these changes are largely within the experimental error. Treatment at 48°C reduces this count at a relatively slow rate for the first 15 minutes but at a much faster rate thereafter.

The tumor-initiating ability (TIA) of heat-treated bacteria (Fig. 2) decreases with time of treatment. The rate of decrease increases as the temperature is raised. Although there is considerable error in these measurements, the TIA decreases without systematic deviation from a simple logarithmic fashion with increase in time at each temperature. These results suggest that a first-order, unimolecular event is responsible for the loss in TIA. The data are relatively unambiguous at the three lower temperatures studied, where few or no cells are killed. At 48°C, the temperature at which cells are killed, the rate of TIA inactivation is, if anything, less than expected (see Fig. 3) from the results at lower temperatures. Since the two measurements are not additive, it appears that cells that have lost TIA are the first to die.

In Fig. 3 the first-order rate constants for TIA inactivation at various temperatures are plotted against the reciprocal of the absolute temperature. The rates from the three lower temperatures fall more or less on a straight line; the value for the 48°C rate is about 35 percent low. From the slope of this line, the heat of activation ($\Delta H\ddagger$), entropy of activation ($\Delta S\ddagger$), and free energy of activation ($\Delta F\ddagger$) for the thermal inactivation of TIA may be calculated (*6*). The resulting values are: $\Delta H\ddagger = 56.7$ kcal mole^{-1}, $\Delta S\ddagger = 107$ cal mole^{-1} deg^{-1}, and $\Delta F\ddagger = 23$ kcal mole^{-1} at 42°C. Values for $\Delta H\ddagger$ of this magnitude are characteristic of heat-induced protein denaturations and of the heat inactivation of virus nucleoproteins (*6, 7*). The $\Delta S\ddagger$ and $\Delta F\ddagger$ values are also in accord with this comparison. The possibility that these data apply to an inactivation of either a free RNA or DNA tumor-inducing principle is remote since studies of heat inactivation of RNA show relatively low $\Delta H\ddagger$ values and negative $\Delta S\ddagger$ values (*8*), whereas thermal denaturation of DNA typically occurs only at much higher temperatures (*9*).

The difference between our $\Delta H\ddagger$ and that observed by Braun (*3*) for the bacteria-host system cannot be considered very significant because the medium in which studies of this kind are performed may alter the observed ΔH‡ value considerably. The factor inactivated in our experiments may be the same as that observed by Braun; may be different, but also associated with the bacterium; or may be, in Braun's work, a critical host component. The relatively high value of $\Delta H\ddagger$ in each case and the single inactivation rate at each temperature, however, suggest that a change in a single protein or nucleoprotein is responsible for the decreased virulence. Apparently this change in virulence does not result in a permanent genetic alteration of the bacterium. A number of individual colonies have been isolated from cultures exposed to temperatures sufficient to reduce infectivity by 80 percent or more. When subcultured and tested for virulence all appear essentially equal in virulence to the untreated parental strain. If the site of action is a nucleoprotein, then either the inactivation process is reversible or two or more sites exist in each bacterium, and the inactivation of one site is sufficient to render the bacterium nonpathogenic.

These values of $\Delta H\ddagger$ and $\Delta S\ddagger$ may be attributable to the "tumor-inducing principle" postulated by Braun (*2*). The data, however, may also be attributed to a component necessary for the production of this principle or for its transfer from bacterium to host cells.

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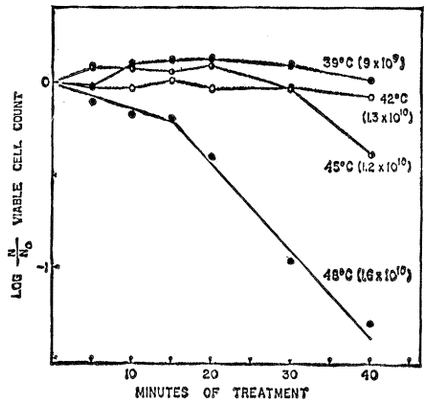

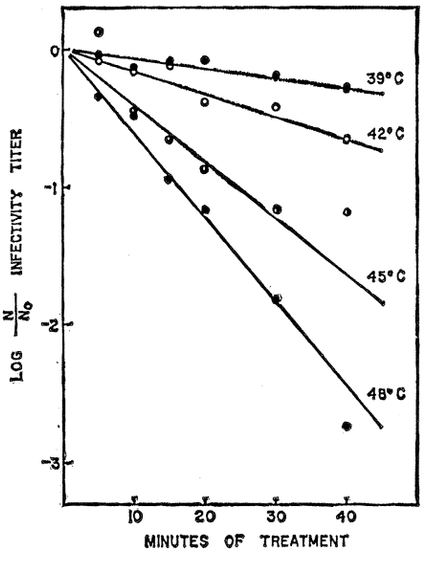

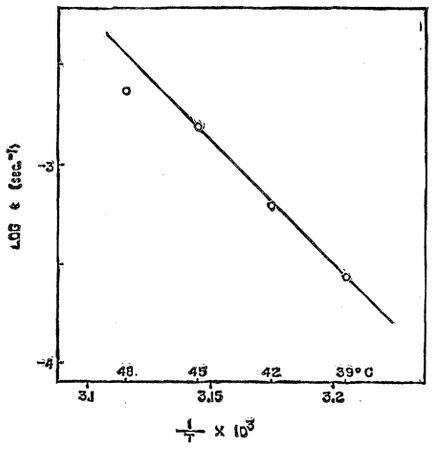

Fig. 1 (top left). Effect of heat treatment at 39° to 48°C on the viability of *Agrobacterium tumefaciens*. The numbers in parentheses are the initial concentrations of viable bacteria per milliliter in the untreated cultures used for the experiments.

Fig. 2 (bottom left). The effect of heat treatments at 39° to 48°C on the tumor-inducing ability of cultures of *Agrobacterium tumefaciens*. Each point is a mean value of determinations from two separate experiments.

Fig. 3 (top right). Temperature dependence of the thermal inactivation rate derived from the data in Fig. 2 for the loss in tumor-initiating ability of *Agrobacterium tumefaciens*.

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