The ability of solutions of ribonuclease to alter the form and mitotic distribution of chromosomes in root-tip cells has been reported in previous publications (KAUFMANN et al. 1952, 1953; KAUFMANN and Das 1954). Production of aberrations by ribonuclease is associated with the degradation of ribonucleoproteins, and this relationship emphasizes the importance of compounds containing ribonucleic acid (RNA) in determining normal chromosome form and distribution in meristematic cells. Because of the implications of these findings with respect to the production of aberrations by other agents, it seems desirable that the observational evidence of the action of ribonuclease on dividing cells be presented in more detail than heretofore. The present report is concerned, therefore, with the effects of solutions of ribonuclease on growing roots of onion (Allium cepa L.) and lily (Lilium tigrinum Ker.). A few confirmatory observations on root tips of the broad bean (Vicia faba L.) are also included.

Materials and Methods.

Onion bulbs and bean seeds were rooted in moistened, sterilized sand, or in tap water, at room temperature (ca. 2² C.). The lily roots were grown from bulbils placed on wet filter paper in petri dishes, which were kept in a 25⁰ incubator. Efforts to root these materials directly in solutions of ribonuclease were not successful, since growth under such conditions was either inhibited or sporadic and slow.

For treatment, the roots were immersed in solutions of ribonuclease or other materials that served as controls. Some of the lily roots were inundated with these liquids without removal from the petri dishes, whereas others were transferred to Columbia staining dishes partitioned by blank cover slips that held up the bulbils and permitted the roots to extend down into the solution. Narrow-throated containers with flaring tops were used to support the onion bulbs and bean seeds, from which the roots depended into the treatment chamber. In an effort to avoid disturbance that might result from uprooting and transfer to liquid medium, the bulbs and seeds were wetted, while growing in sand, with the solutions to be tested, but this method of treatment gave such variable results that it was soon discarded.

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Solutions in which the roots were immersed were aerated continuously during the course of treatment, since preliminary tests had shown that exhaustion of dissolved air from water in which roots were growing led to production of abnormalities with a frequency in excess of that found in roots removed directly from bulbs and seeds planted in sand.

In the course of these studies seven different samples of ribonuclease were tested. They were extracted from beef pancreas, crystallized, purified, and assayed for enzymatic activity in this laboratory by Dr. Margaret McDonald, using her modification (1948) of the method of Kunitz (1940). All were effective in producing abnormalities of the types described in the next section of this article.

These samples were used experimentally in concentrations ranging from 0.001 to 1.0 mg. per ml., dissolved in water or 0.01 M potassium phosphate buffer. When distilled water was used as the solvent, the pH of the solution was about 5.8. Solutions of ribonuclease in tap water gave pH's close to 6.0, and could be adjusted to that pH with a trace of 0.001 N sodium hydroxide. Care was necessary, however, to avoid excessive concentrations of the alkali, which made the roots translucent. For that reason, treatments at pH's higher than 6 were made with solutions of ribonuclease in phosphate buffer. Duration of treatment varied in different experiments from 15 minutes to 48 hours. Following exposure to the action of the enzyme, the roots were transferred to tap water or phosphate buffer to determine the sequelae of the treatment.

An extensive series of controls was maintained. It is customary in studies involving chemical treatment of cells to regard the solvent as an adequate control on the solute being tested, but the use of enzymes requires evaluation of other variables. The theoretically ideal control in experiments employing ribonuclease would be the enzyme in an inactive but otherwise unaltered state. Inactivation may be effected by heating the enzyme solution or exposing it to ultraviolet radiation, but these methods not only denature the protein molecules but also alter other properties of the solution. In assessing the validity of inactivated ribonuclease as a control it was necessary, therefore, to determine whether the products of denaturation, or the substances produced by irradiation of the solvent were in themselves capable of modifying mitotic processes in root-tip cells. It was also necessary to evaluate the action of products arising from the degradation of RNA by ribonuclease and the effect on dividing cells of solutions of enzymatically inactive substances having molecular weights and osmotic properties comparable with those of the ribonuclease solutions.

On the basis of the foregoing requirements, the controls included the following solvents and solutes: unirradiated and ultraviolet irradiated tap water and distilled water, potassium phosphate buffer, inactivated ribonuclease (prepared from a 1 mg. per ml. solution that had been boiled or exposed to ultraviolet radiation of wavelength 2537 Å, which reduced the activity, as determined by chemical assay, to less than that of a solution containing $1.25 \times 10^{-4}$ mg. per ml.), a mixture of amino acids in the proportions characteristic of ribonuclease (Brand, 1948), histone from calf thymus, protamine from salmon, egg albumin, sucrose, and a ribonuclease digest of RNA. In addition, crystalline deoxyribonuclease, trypsin, and chymotrypsin were tested to determine whether these enzymes evoked the same types of mitotic abnormalities as ribonuclease. All the solutes were used in concentrations equimolar with ribonuclease under parallel conditions of treatment.


2 The sample of protamine used was generously supplied by Eli Lilly and Co. to whom the authors wish to express their thanks.