Plant Growth Inhibition by Streptomycin and Its Prevention by Manganese. (20889)

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The literature concerning streptomycin (SM) effects in plants has been reviewed (1,2). Exposure to suitable concentrations of SM has been reported to result in two general effects: inhibition of growth and "bleaching." In higher plants growth inhibition resulting from SM treatment consists of inhibition of leaf expansion, of root growth, and of stem elongation. Bleaching refers either to the loss of chlorophyll from green tissue or to the failure of potentially chlorophyllous tissue to become green after exposure to SM. Both bleaching and growth inhibition have been reported to occur after treatment of certain phytoflagellates(3,4), germinating seeds of various monocots(1,5,6), carrot tumor tissue (7), and seedlings of radish(8), and bean(1).

As a means of investigating the growth inhibiting action of SM an effort has been made to reverse or prevent this inhibition by the application, in conjunction with the SM treatment, of various metabolites and other compounds which might enhance growth of plant tissue.

Materials and methods. For this purpose the effect of SM on the growth (cell elongation) of *Avena* coleoptile sections has been studied. The seedlings (*Avena sativa* var. Swedish victory) were grown in the dark as for the standard *Avena* test. After approximately 72 hours a section 5.3 mm in length was cut from near the apex of the coleoptile of each seedling with a double-bladed cutter, and 10 such sections were floated on 10 ml of each solution to be tested, in 125 ml Erlenmeyer flasks. The experiments were conducted in a dark-room at 24-25°C and 85-90% relative humidity. Red light was used for manipulations. After 24 hours the sections were removed from the solutions and their length measured with an ocular micrometer in a binocular dissecting microscope. All test solutions contained 2% sucrose. Indole-3-acetic acid (IAA) was supplied in different concentrations within the optimal range for growth promotion (1-10 mg/l). No appreciable difference in the SM inhibition of growth or in its reversal was observed within this IAA range. In each experiment the controls were supplied with IAA and sucrose only. SM concentrations are expressed as mg of SM base per liter. SM and dihydro SM sulfates were equally effective. The average increase in length of the sections in the various test solutions is expressed as a per cent of the increase in length of the controls. The mean elongation of the controls, which were initially 5.3 mm long, varied from 3.5 to 5.5 mm.

Results. Fig. 1 shows the effect of increasing concentrations of SM. Low concentrations (0.001-1.0 mg/l) caused a slight but variable increase in elongation. All SM concentrations above 1 mg/l which were tested

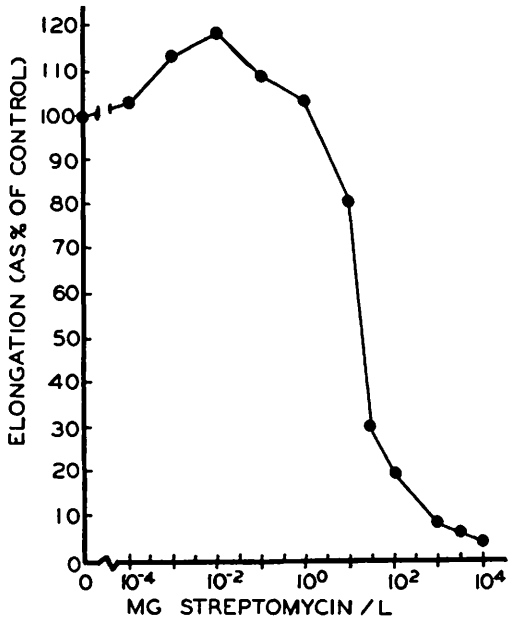

FIG. 1. Effect of streptomycin concentration on elongation of *Avena* coleoptile sections. (Sucrose, 2%; IAA, 1-10 mg/l; each point based on 1 to 9 experiments.)

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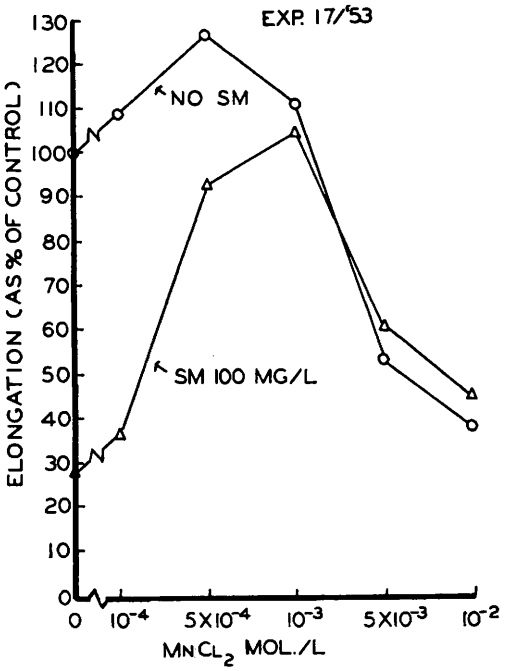

FIG. 2. Effects of manganous ion concentration on elongation of *Avena* coleoptile sections in presence and absence of streptomycin. (Sucrose, 2%; IAA, 1 mg/l.)

inhibited elongation. Ten and 100 mg SM/l caused decreases of about 20 and 80%, respectively, but even with 100 mg SM/l the sections retained normal turgor, indicating that despite the drastic reduction in growth, they were not killed. For this reason, 100 mg/l was the SM concentration employed in most experiments.

Since the Mn^{++} ion enhances seedling growth(9) and has been shown to enhance the elongation of *Avena* coleoptile sections in the presence of IAA and sucrose(10), the effect of serial concentrations of this element was tested in the presence and in the absence of SM. A marked effect on elongation was noted with both manganous chloride and sulfate. Inasmuch as other chloride and sulfate salts were without comparable activity, the effect appears to be due entirely to the Mn^{++} ion. The results of a typical experiment are shown in Fig. 2. It will be noted that throughout the stimulatory range of Mn^{++} concentrations the effect of the element was considerably greater in the presence of SM than in its absence. This indicates that the effect of the Mn^{++} ion in the presence of SM is not merely a general enhancement of growth. In 5 separate experiments the optimal concentration of Mn^{++} (10^{-3} M) allowed growth of the SM-treated sections which averaged 106.8 $\pm$ 4.6% of that of the controls. Higher concentrations of Mn^{++} were inhibitory, also in the absence of SM. Typical average increases in length, with standard errors, obtained in the presence of either 100 mg SM/l, or 10^{-3} M Mn^{++}, or both, are shown in Table I.

The inhibition resulting from SM concentrations in excess of 100 mg/l was at least partially prevented by simultaneously applied high concentrations of Mn^{++}. However, complete protection from inhibition by the highest levels of SM employed (500 and 1000 mg/l) could not be obtained, possibly because of osmotic and/or toxic effects of the high Mn^{++} levels themselves. These points are illustrated in Fig. 3.

Chloride salts of other cations have been tested for activity in preventing SM inhibition of coleoptile elongation. Na^{+}, Mg^{++}, Ni^{++}, and Co^{++} were without effect. Ca^{++} appeared to be about 50% as effective as Mn^{++}. Although K^{+} itself was without effect, the effects of Ca^{++} or Mn^{++} were increased in its presence. Mn^{++} was effective in preventing SM inhibition also in the presence of various combinations of cations, including dilute major element nutrient solution.

Various sugars, amino acids, and Krebs

TABLE I. Growth (in mm) of *Avena* Coleoptile Sections Exposed to Mn^{++} (10^{-3} M) and Streptomycin (100 mg/l), Alone and in Combination; (Sucrose, 2%; IAA, 1 mg/l; Initial Length of Sections, 5.3 mm).

Increase in length	No streptomycin		Streptomycin, 100 mg/l	
	No Mn	Mn 10^{-3} M	No Mn	Mn 10^{-3} M
Avg	4.0 $\pm$ .1	4.9 $\pm$ .2	.9 $\pm$ .1	4.4 $\pm$ .3
Rel. (%)	100 $\pm$ 2.5	123 $\pm$ 5.0	22 $\pm$ 2.5	110 $\pm$ 7.5

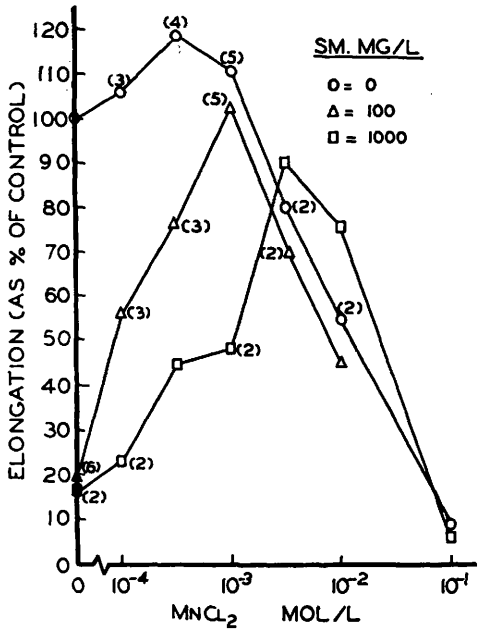

FIG. 3. Effect of manganous ion concentration on elongation of *Avena* coleoptile sections in presence of 2 concentrations of streptomycin. (Sucrose, 2%; IAA, 1-10 mg/l; numbers in parentheses indicate number of experiments upon which each value is based.)

cycle acids, as well as Coenzyme A and adenine, were without significant effect in preventing the SM inhibition.

Experiments were conducted to determine the effect of exposure of the sections to SM on their subsequent growth in Mn^{++} solutions, and *vice versa*. Marked inhibition resulted when the sections were treated with 100 mg SM/l for as little as one hour. This inhibition was not reduced significantly when the sections were transferred from SM to a solution containing Mn^{++}. Pretreatment with Mn^{++} appeared to result in a slight reduction of SM inhibition, however.

The effect of Mn^{++} on the bacteriostatic action of SM has been examined in cultures of *Escherichia coli* "B". The bacteria were grown in nutrient broth containing 0.5% sodium chloride, and growth was measured as change in optical density. It was found that these cultures were inhibited equally in the presence and absence of Mn^{++}.

Discussion. The observation that the bacteriostatic action of low concentrations of SM is not affected by the presence of relatively high concentrations of Mn^{++} suggests that, in preventing SM inhibition of the growth of higher plant tissue, the action of Mn^{++} is upon cellular processes rather than a reaction with the SM molecule itself. This ability of Mn^{++} to reduce growth inhibition of higher plants by SM without affecting bacteriostasis may prove to be of value in the control of bacterial plant pathogens with SM. The results do not reveal the nature of the interaction of Mn^{++} and SM. The rise in optimal Mn^{++} concentration with increasing levels of SM suggests competition between the 2 substances. If this is so the inefficacy of pre- and post-treatments with Mn^{++} would indicate that SM has a vastly greater affinity than Mn^{++} for the reactive site. This is not supported by the relatively low Mn^{++}/SM mole ratio which prevents SM inhibition in combined treatments. Other possibilities consistent with the present findings are: 1) that Mn^{++} activates an alternative, SM-insensitive metabolic pathway in the higher plant tissue which is not present in the tested strain of *E. coli*, and 2) that Mn^{++} prevents the entry of SM into the higher plant cell. Experiments designed to test these hypotheses are now in progress.

Summary. The marked growth inhibition of *Avena* coleoptile sections caused by 100 mg streptomycin/l was prevented when manganous ions were added to the test solution. Pretreatment with Mn^{++} had only a slight effect on SM inhibition, and post-treatment was ineffective. Other cations and various organic compounds were without activity comparable to that of Mn^{++} in preventing the streptomycin inhibition of growth. Mn^{++} did not reduce streptomycin inhibition of the growth of *E. coli* "B" in liquid culture.

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An Antihemolytic Action of Pyridoxal.* (20890)

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In studying the action of pyridoxal on the concentrative uptake of amino acids by the Ehrlich mouse-ascites tumor cell, an antihemolytic action toward the contaminating red blood cells was accidentally noted. The cause of the spontaneous hemolysis in the ascitic fluid *in vitro* is unknown, but samples to which mM pyridoxal had been added escaped red cell breakage. Although we have not been able to demonstrate at will a protective action of pyridoxal at this level, an antihemolytic action can readily be shown at a 10 mM level.

Method. The procedure used was much like that of Wilbrandt(1). Blood was taken by venipuncture from persons in the postabsorptive state, and the blood treated with heparin. Aliquot portions were added to 2 samples of 10 volumes of the medium of Raker *et al.*(2). In one of the 2 portions of medium 10 mM per L of pyridoxal, or of sodium indoleacetate, replaced 5 or 10 mM per L of sodium chloride, respectively; in this way the osmotic pressure was kept uniform. The suspensions were then shaken for one hour at 37°C in an atmosphere of 95% oxygen and 5% carbon dioxide. Aliquot portions were then added with stirring to 10 to 20 volumes of hypotonic sodium chloride, buffered at pH 7.4 with 15 mM sodium phosphate. The suspensions were stirred occasionally for an hour, then centrifuged and the extent of hemolysis determined by measuring spectrophotometrically the optical density of the supernatant hemoglobin solutions. The time intervals were not critical; the same results were obtained after 15 minutes of exposure to hypotonicity. In calculating the osmolarities of the hypotonic solutions account was taken of the volume of the Raker medium and plasma included in the mixture.

Results and discussion. The results are illustrated in Fig. 1. The shift of the position of the curve was by 5 to 10 milliosmoles per liter. This effect would be expected if pyridoxal caused a displacement of cell solutes or a shrinkage of the cells. With the carcinoma cells of the Ehrlich ascites tumor, pyridoxal causes large amounts of potassium to move out of the cells; this shift is partially compensated by chloride exodus, and partially by sodium entrance into the cells(3). The

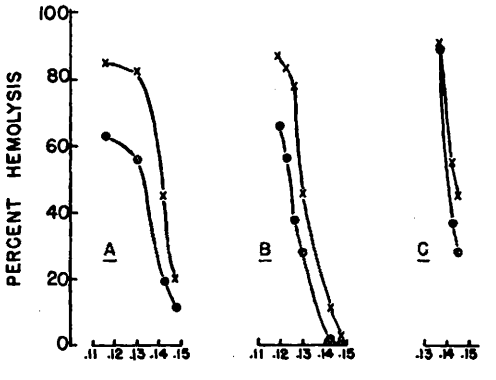

FIG. 1. Antihemolytic action of 10 mM pyridoxal (A and B) and indoleacetate (C). The encircled points represent the degree of hemolysis when the chemical agent was present, the crosses, when it was absent.

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