Technical Papers

Translocation of Streptomycin in Bean Plants and its Effect on Bacterial Blights

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Primary leaves of bean inoculated with the halo blight organism *Pseudomonas medicaginis* var. *phaseolicola* (Burk., Stapp and Kotte) failed to develop symptoms of halo blight when a minute amount of streptomycin[1] sulfate was placed on the stems of the plants prior to inoculation. Similar plants inoculated in a like manner developed very mild symptoms of the disease when dihydrostreptomycin sulfate was applied to their stems prior to inoculation. These antibiotics were apparently absorbed by the stems and translocated upward into the primary leaves in sufficient amounts to prevent growth and development of the organism. Of 12 antibiotics tested, streptomycin sulfate and dihydrostreptomycin sulfate were highly effective against this organism (Table 1). Streptomycin sulfate did not affect growth of the plants a detectable amount, but dihydrostreptomycin sulfate checked their growth very slightly at the dosage level used.

In testing the antibiotics, a 1% mixture of each was made by dissolving 12.5 mg of the compound in 0.25 g of Tween 20[2] and then adding 1 g of melted lanolin. The mixture was warmed and then stirred vigorously while it cooled. A small portion of the paste containing approximately 0.2 mg of the antibiotic was applied as a thin layer covering the internode immediately above the cotyledons of each plant. Ten seedlings of the Black Valentine variety grown in a greenhouse were used in testing each antibiotic. At the time of treatment their primary leaves were about 2 in. across and their trifoliate leaves were still folded in the terminal buds.

Three days after the antibiotics were applied, the upper surfaces of the primary leaves of each plant were dusted lightly with No. 320 powdered carborundum and then rubbed with the bacterial suspension. A beef broth culture was incubated for about 48 hr and then diluted with water at the rate of 1 : 10. An equal number of comparable plants to which no antibiotic had been applied were inoculated for comparison. Primary leaves of these untreated plants developed marked symptoms of the disease within 5–7 days after inoculation, whereas plants to which streptomycin sulfate had been applied failed to develop symptoms. This experiment was done 3 times with similar results each time.

Comparable tests using the 12 antibiotics on plants inoculated with *Xanthomonas phaseoli* (E. F. Sm.) Dowson (the common blight organism) demonstrated that dihydrostreptomycin sulfate and streptomycin sulfate were absorbed by the stems and translocated to the primary leaves of bean plants in amounts which prevented the production of symptoms of common blight without appreciably affecting growth of the test plants. Terramycin hydrochloride and aureomycin hydrochloride reduced the severity of symptoms but injured the plants and suppressed growth. The other antibiotics did not reduce severity of symptoms appreciably.

TABLE 1

DEGREE OF LEAF INFECTION OF BEANS WITH THE HALO BLIGHT ORGANISM AFTER TREATMENT OF THE STEMS WITH VARIOUS ANTIBIOTICS

Antibiotic	Degree of infection
Streptomycin sulfate	None
Dihydrostreptomycin sulfate	Slight
Terramycin hydrochloride	Moderate
Neomycin sulfate	Marked
Bacitracin, topical	"
Antibiotic Q-19	"
Aureomycin hydrochloride	"
Threo D-chloromycetin	"
Polymixin	"
Tyrothricin	"
Subtilin	"
Penicillin G potassium	"

Streptomycin sulfate was absorbed by the stems of bean seedlings and translocated to trifoliate leaves 6–8 in. from the point of treatment in sufficient amounts to reduce the incidence of infection and severity of symptoms produced by the halo blight organism. In this experiment, 0.2 mg of the antibiotic in the form of the paste previously described was applied in a band around the first internode of 8 plants. A comparable number of untreated plants were used as controls. The trifoliate leaves were folded in the terminal buds at the time the antibiotic was applied. Later, when they were approximately 2 in. long, leaflets of the first and second trifoliate leaves were inoculated with the halo blight organism as previously described. All inoculated leaves on the untreated plants showed marked symptoms of the disease within 1 week after inoculation. Forty-five per cent of the first trifoliate leaves of plants treated with the antibiotic showed no symptoms, and the remainder developed slight symptoms. All the second trifoliate leaves had developed symptoms at the end of this period, 67% of them showing mild symptoms and the remainder moderately severe ones.

[1] Streptomycin sulfate and dihydrostreptomycin sulfate supplied by Charles Pfizer and Co., Inc., Brooklyn, N. Y.; chloromycetin by Parke Davis and Co., Detroit, Mich.; the remaining antibiotics by Food and Drug Administration.

[2] A sorbitol derivative obtainable from Atlas Powder Co., Wilmington, Del.

Streptomycin sulfate applied as a water solution at the rate of 1, 3, and 9 lbs of antibiotic/acre to soil in which bean seedlings were growing failed to reduce the incidence of infection or severity of symptoms that developed on their primary leaves inoculated with the halo blight organism.

Bean plants treated with streptomycin sulfate failed to accumulate a sufficient amount of the antibiotic in their seeds to affect the susceptibility to the halo blight organism of seedlings that developed from these seeds. In this experiment, the antibiotic was applied as the paste previously described to those internodes of relatively mature plants which subtended fruit clusters. Other plants were sprayed twice with an aqueous solution containing 0.1% of streptomycin sulfate. Pods approximately 2–3 in. long at the time of treatment were harvested at maturity; the seeds in these were planted, and the first trifoliate leaves of the resulting seedlings were inoculated with the halo blight organism. There was no apparent difference between the severity of symptoms that developed on seedlings from treated and on those from untreated parent plants.

Several investigators have shown that roots of plants such as oats, lettuce, soybean, and lima bean can absorb antibiotics such as aureomycin, streptomycin, and griseofulvin from aqueous solutions of these compounds and translocate them to the leaves and stems (*1–3*). Boyle (*4*) reported that penicillin injected into cactus stems diffused into near-by infected areas and killed *Erwinia carnegieana* Lightle, Standring, and Brown. Others (*5, 6*) have reported that streptomycin, neomycin, bacitracin, chloromycetin, subtilin, and penicillin reduced the prevalence of halo blight when infected bean seeds were soaked in aqueous solutions of these antibiotics before planting.

Absorption and translocation of an antibiotic by an aboveground plant part in sufficient amounts to retard or inhibit a bacterial disease have not been reported previously. It is concluded from the experiments reported here that dihydrostreptomycin sulfate and streptomycin sulfate were absorbed by the stems of bean seedlings and translocated upward to the primary leaves where, within a period of 3–4 days, they accumulated in sufficient amounts to inhibit or prevent the growth and development of the halo and common blight organisms. Within a week after application of streptomycin sulfate to the stems of bean seedlings, a sufficient amount of the chemical had been absorbed and translocated to the first and second trifoliate leaves to suppress development of the halo blight organism.

References

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Metabolism of Δ^1-Androstene-3,17-Dione

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Δ^1-Androstene-3,17-dione has been isolated from pooled urines from healthy and diseased individuals (*1*). Saturation of ring A has been shown to occur when this steroid is incubated with fermenting yeast (*2*), since the product androstane-3β,17β-diol was isolated. In the present study we have demonstrated that the oral administration of Δ^1-androstene-3,17-dione gives rise to relatively large amounts of androsterone, isoandrosterone, and $\Delta^{2 \text{ or } 3}$ androstene-17-one in the urine (Table 1). The $\Delta^{2 \text{ or } 3}$-androstene-17-one is considered to arise from androsterone, as a result of the chemical procedures employed during isolation (*3–5*).

TABLE 1

URINARY METABOLITES OF 3.2 G Δ^1-ANDROSTENE-3,17-DIONE FOLLOWING ORAL ADMINISTRATION

Compound isolated	Amount isolated (mg)	Melting points in ° C*			Percentage isolated
		Free	Acetate	Oxime	
Androsterone	210	184–5.5	163–4		10.9
	140	181–3	163–4		
$\Delta^{2 \text{ or } 3}$-Androstene-17-one	125	104–6.5		156–8.5	3.9
Isoandrosterone	55	175–7	115–7		1.7

* Fischer-Johns apparatus—uncorrected.

Over a 60-hr period, 3.2 g of Δ^1-androstene-3,17-dione[2] was administered orally in capsules to an adult male with rheumatoid arthritis. The control value for 17-ketosteroids for this individual was 9.8 mg/24 hr. The urine was collected during the treatment period and for 36 hr after treatment was stopped. The urine was extracted with butanol by the method of Venning (*6*), the butanol evaporated to dryness, and the residue subjected to simultaneous hydrolysis with HCl and extraction with benzene. The ketonic and nonketonic fractions were prepared using Girard's reagent T. The ketonic fraction was chromatographed, using aluminum oxide as adsorbant, and eluted with carbon tetrachloride, followed by carbon tetrachloride containing 0.1, 0.2, and 0.3% ethanol, respectively.

The ketonic fraction upon elution with carbon tetrachloride yielded 125 mg $\Delta^{2 \text{ or } 3}$-androstenone-17. Further elution with carbon tetrachloride containing 0.2% ethanol yielded 350 mg androsterone. The β-ketonic fraction precipitated with digitonin yielded 55 mg iso-

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[2] A gift from the Chemical Specialties Co., Inc., New York City.