These observations suggest that *Octopus bimaculoides* and *O. bimaculatus* may prey naturally on shelled mollusks by drilling a hole and injecting venom. We have found in nature numerous empty shells of the species *Tegula funebralis* Adams, *Chione undatella* Sby., *Protothaca staminea* Conrad, and *Haliotis* spp. with holes identical in shape to those made by *Octopus* in the aquarium (*9*).

Note added in proof: Dr. S. Stillman Berry has brought a paper by Fujita (*10*) to our attention. Fujita discovered that *O. vulgaris* on the coast of Japan bored holes into the shells of the pearl oyster, and he suggested that venom was injected to weaken the adductor muscle.

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Translocation of Streptomycin from Coleus Leaves and Its Effect on Rhizosphere Bacteria

Abstract. Autoradiographs showed that when streptomycin is applied to leaves of coleus plants (1000 to 3600 micrograms per plant) the antibiotic or some by-product is translocated laterally and downward. The translocated material does not alter rhizosphere microorganisms quantitatively. Gram-negative rhizosphere bacteria, however, are suppressed through the 8th day after treatment.

In studies of the ecology of rhizosphere microorganisms, it became of interest to produce selective quantitative and qualitative alterations in the populations of these organisms in their natural habitat. Two general approaches are available. The first involves treating the entire soil since the location of the rhizosphere cannot be determined beforehand. The second involves application of an active material to the plant (or seed) in a manner that will permit downward translocation of the active material to the roots. The second approach has been used previously (*1*). When Bordeaux mixture, a nonselective agent, was applied to leaves of beans, it increased the levels of copper and reduced the numbers of bacteria in the plant rhizosphere.

The possibility of utilizing selective materials of complex molecular structure was tested by applying streptomycin to leaves of coleus plants (*Coleus blumei* Benth) whose rhizosphere microflora was then periodically sampled. Streptomycin and coleus were selected because (i) this antibiotic apparently moves downward in coleus stems (*2*), (ii) it persists in some plants for as long as 8 weeks (*3*), and (iii) it is degraded relatively slowly in soil (*4*). Also we found that streptomycin is not phytotoxic to coleus at levels of application in excess of 4000 μg per plant.

The movement of antibiotics in plants apparently varies with the plant and the antibiotic employed (*5*). In cherry trees (*6*) and peach trees (*7*) streptomycin moves very little or not at all from treated leaves. In hops (*8*) and beans (*9*) it moves upward with ease, in detectable amounts when applied at low dosages, but not downward. In apples and pears it moves both upward and downward when applied at relatively high dosages (*10*).

All the plants we used were grown in unsterilized soil from split-node cuttings from a single parent plant. The plants were of uniform age and size and generally had four to six fully developed leaves.

Streptomycin sulfate at 10,000 μg/ml of 1-percent glycerol was applied, at rates which varied in the several experiments from 0.03 to 0.10 ml per application, in a band across the widest part of the leaf or leaves. The mixture of antibiotic and glycerol was then spread with a thin glass rod over the upper leaf surface except near the petiole. This method prevented any accidental external movement of the streptomycin down the petiole to the stem. Material in excess of that contained in 0.10 ml of solution was applied to the leaves in equal portions as described above. The leaves were allowed to dry between applications to prevent dripping from the leaf tip.

Table 1. Effect of streptomycin applied to leaves of coleus on the percentage change in numbers of Gram-negative rhizosphere bacteria at different times after treatment. In test 1, 1000 μg of streptomycin sulfate were applied to each plant in one application; in test 2, 3600 μg of streptomycin sulfate were applied to each plant in three applications.

Time after treatment (day)	Change in Gram-negative rhizosphere bacteria (%)		
	Test 1	Test 2	Mean
0	0	0	0
4	−37	−20	−28
8	−22	−22	−22
12	−9	+9	0

The results of a series of experiments are reported here. In the first experiment 1000 μg of streptomycin sulfate were applied to each plant in one application. In the second, 3600 μg were applied to each plant in three applications of 1200 μg each. In both cases the treatment was equally divided between the two leaves arising at a single node. Control plants were treated identically with 1-percent glycerol solution without streptomycin. Four replications were used throughout.

The plants were carefully lifted from the soil 4, 8, and 12 days after treatment, and the superfluous soil was removed from the roots by tapping the plant sharply several times. The roots and adhering soil were immediately transferred to sterile water blanks. After rotary agitation for 30 minutes, dilutions were prepared and dilution plates were made by adding 10 ml of agar medium to 1 ml of the final suspension in a petri plate. Bacteria and streptomycetes were enumerated on egg-albumin agar, and fungi were enumerated on a vegetable juice–dextrose–yeast extract agar containing antimicrobial agents (*11*). The total numbers of the three groups of microorganisms did not

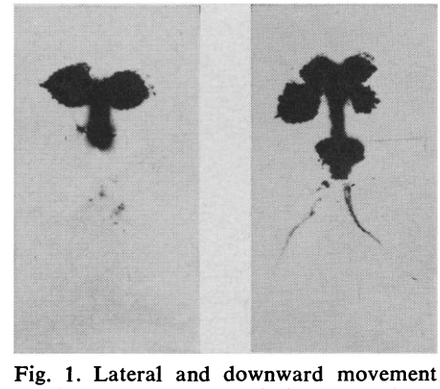

Fig. 1. Lateral and downward movement of C^{14}-labeled streptomycin in coleus plants 6 hours (left) and 24 hours (right) after application of 3000 μg of labeled streptomycin to the upper left-hand leaf of each plant (about × 1/5).

change appreciably in the rhizosphere.

In addition to counting the organisms at each harvest time, we picked at random 100 bacterial colonies from each group of plants (treated and control), and prepared Gram stains. In both experiments a strong suppression of Gram-negative bacteria was observed 4 and 8 days after treatment (Table 1). The effect largely disappeared by the 12th day.

Although foliar application of streptomycin resulted in an appreciable shift in the ratio of the Gram-negative to the Gram-positive bacteria in the coleus rhizosphere, the results do not necessarily prove that streptomycin was translocated downward.

Several bioassays were made with coleus plants treated on one leaf only to trace the movement of streptomycin. These tests included direct plating of leaf, petiole, stem, and root segments; freezing and thawing the various plant parts and then pressing out the plant juice and applying samples of this juice to sensitivity disks; and growing the plants in liquid culture, vacuum-concentrating the liquid at 35°C to a 40-times concentration, and applying samples of the concentrated liquid both to sensitivity disks and in glass wells. All tests were run on nutrient agar seeded with a streptomycin-sensitive strain of *Bacillus subtilis* Cohn. The results of these tests indicated movement of the streptomycin or some antimicrobial metabolite of it from the treated leaf to the opposite leaf, to the internodal stem section below the treated leaf, and to the leaf directly below the treated one. No tissues above or below those mentioned gave any indication of the presence of streptomycin. Sampling times varied between 6 hours and 6 days after treatment. These results may indicate either that the streptomycin is distributed in the plant to a limited extent or that the material remaining free and active after "binding" (*7*) by plant substances is present in minute quantities not detectable by conventional bioassays.

Resolution of this problem became possible through the use of C^{14}-labeled streptomycin (*12*). Eight uniform coleus plants about 6 inches tall were selected, and one leaf about midway up the stem on each of four of the plants received 3000 μg of a calcium chloride complex of labeled streptomycin in 1-percent glycerol possessing an activity of 0.054 μc/mg. Therefore, each plant received 0.162 μc. The remaining four plants received distilled water containing 1-percent glycerol only. Two streptomycin-treated and two control plants were harvested 6 hours after treatment and the remaining four after 24 hours. Soil was washed from plant roots, and each plant was then placed on a sheet of blotting paper. The roots were cut from the stem to prevent any further movement of streptomycin, a second sheet of blotting paper was placed on the plant, and the mounted plant was immediately placed in a freezer. When the plants were completely frozen, x-ray film was placed on the plants in light-proof holders. A thin window counter indicated that the minimum satisfactory exposure of the treated plant was 30 to 60 days. On the basis of these results an exposure of 75 days was selected. The autoradiographs (Fig. 1) show that carbon-14 is translocated from the treated leaf laterally and downward to the tips of the roots within 24 hours. The upper left-hand leaf in both radiographs was the one treated with labeled streptomycin. The treated leaf was approximately in the middle of the stem. The stem and leaves above the treated leaf produced no image on the radiographs. This unidirectional movement is in contrast to the results obtained by other workers when plants other than coleus were used in studying translocation of streptomycin.

It appears that when streptomycin is applied to leaves of coleus plants in relatively large amounts the antibiotic or some by-product is translocated laterally and downward, but not upward, and acts to suppress the Gram-negative bacteria in the rhizosphere of the treated plants.

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Effect of Molecular Weights of Colloidal Dextran on Human Serum Lipids

Abstract. When 6-percent colloidal solutions of dextran (molecular weights, 10.000 to 500.000) are mixed with human serum in vitro, a new dextran-lipid fraction appears in paper strip electrophoresis between the starting point and the gamma globulins. The intensity of this dextran-lipid fraction increases with the progressive increase of molecular weight of the dextran used, and this increased intensity of the dextran-lipid fraction is accompanied simultaneously by an appropriate decrease of the lipid fraction migrating with the beta globulins. The intensity of the A-lipoproteins and the neutral fats adsorbed at the starting point seems unaltered by the application of dextran in colloidal solution regardless of the molecular weight of the dextran used. No change of the protein patterns was observed.

Keler-Bačoka *et al.* (*1*) recently described the effect of dextran [Macrodex, Uppsala, Sweden (molecular weight, 70.000)] in a 6-percent colloidal solution for infusion use applied in vivo and in vitro on human serum lipids. This effect consists in changes of the lipid pattern in paper strip electrophoresis: Between the starting point and the gamma globulins a new lipid fraction appears with a slower mobility than any mobile protein or lipid fraction in normal serum. This fraction, called by the authors the dextran-lipid fraction, obviously diminishes the intensity of the lipid fraction migrating with the mobility of the beta globulins.

In this report the effect of dextran, in colloidal solution and with increasing molecular weights, on human serum lipids is studied in vitro.

Six-percent colloidal solutions of dextran of different molecular weights (10.000 to 500.000) (*2*) were obtained by dissolving dextran in redistilled water at 95°C. Data for the dextrans used are shown in Table 1.

The sera of 20 adult persons were mixed in vitro with colloidal solutions of dextran of different molecular weights

Table 1. Average molecular weights of the dextrans used, as determined by different methods. The dextrans used were prepared from hydrolyzed dextran through a repeated partial precipitation procedure. See also B. Ingelman and M. Halling, *Arkiv Kemi* **1**, 61 (1949). [Pharmacia, Uppsala, Sweden]

Supposed molecular weight	Light scattering	Analysis of end groups
10.000	10.500	6.100
40.000	41.000	22.300
80.000	75.000	46.200
150.000	153.000	95.000
500.000	450.000	200.000