

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  $\mu$ g of a calcium chloride complex of labeled streptomycin in 1-percent glycerol possessing an activity of 0.054  $\mu$ c/mg. Therefore, each plant received 0.162  $\mu$ c. The remaining four plants received distilled water containing 1-per-

cent 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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12. We are indebted to Dr. H. B. Woodruff, Merck & Co., Rahway, N.J., for supplying the  $C^{14}$ -labeled streptomycin.

19 June 1961

## 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

by mixing 1 ml of serum with 0.5 ml of a 6-percent dextran solution. All the unmixed as well as the mixed sera were run in duplicate on filter paper strip electrophoresis and stained for lipids. The third filter paper strip was run and stained for proteins. The electrophoresis was performed on filter paper (Whatman No. 1) in veronal-acetate or in phosphate buffer at pH 8.6, ionic strength 0.1, in a potential gradient of 3 to 4 volt/cm for 18 hours. The lipid patterns were stained with Sudan Black (3) and the protein patterns with Amido-black (4). The densitometric scannings were performed on an Elphor densitometer.

All lipidograms of the human sera mixed with different molecular weights of dextran in colloidal solution without exception showed the dextran-lipid fraction. It is evident from the lipidograms and their densitometric scannings shown in Fig. 1 that the increase of the molecular weight of the dextran added increases the intensity of the dextran-lipid fraction, and at the same time decreases the intensities of the lipids migrating with the beta globulins. With dextran of molecular weight 500,000, the latter lipid fraction disappears almost completely. The intensities of the A-lipoprotein fraction, as well as of the neutral fats adsorbed at the starting

point (NF), seems unchanged by colloidal solutions of dextran of different molecular weights in vitro.

The use of approximately equimolar concentrations of the colloidal solutions of dextrans with increasing molecular weights was omitted in these experiments, because the choice of a concentration of a dextran solution which shows just a faint effect of the formation of the dextran-lipid fraction would result, in the experiment with the next higher molecular weight of dextran, in complete disappearance of the beta-lipoprotein fraction and maximal formation of the dextran-lipid fraction.

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14 February 1961

### Myelin-Binding Antibodies in Experimental "Allergic" Encephalomyelitis

**Abstract.** Antibodies with a specificity for myelin, as demonstrated by immunofluorescence, are present in the sera of rabbits injected with spinal cord. These antibodies react with both adult rabbit and human spinal cord but not with unmyelinated areas of neonatal human spinal cord.

Complement-fixing antibodies are known to be present in the sera of animals with experimental "allergic" encephalomyelitis (EAE) (1). Moreover, Bornstein and Appel (2) have recently shown that the sera of rabbits with "allergic" encephalomyelitis are capable of producing demyelination of rat cerebellum in tissue culture. The latter finding prompted us to study the sera of such animals for the presence of myelin-binding factors. Fluorescent-antibody studies by Beutner *et al.* (3) suggested that the myelin sheath may be one of the antibody combining sites.

"Allergic" encephalomyelitis was induced in seven of a total of ten rabbits

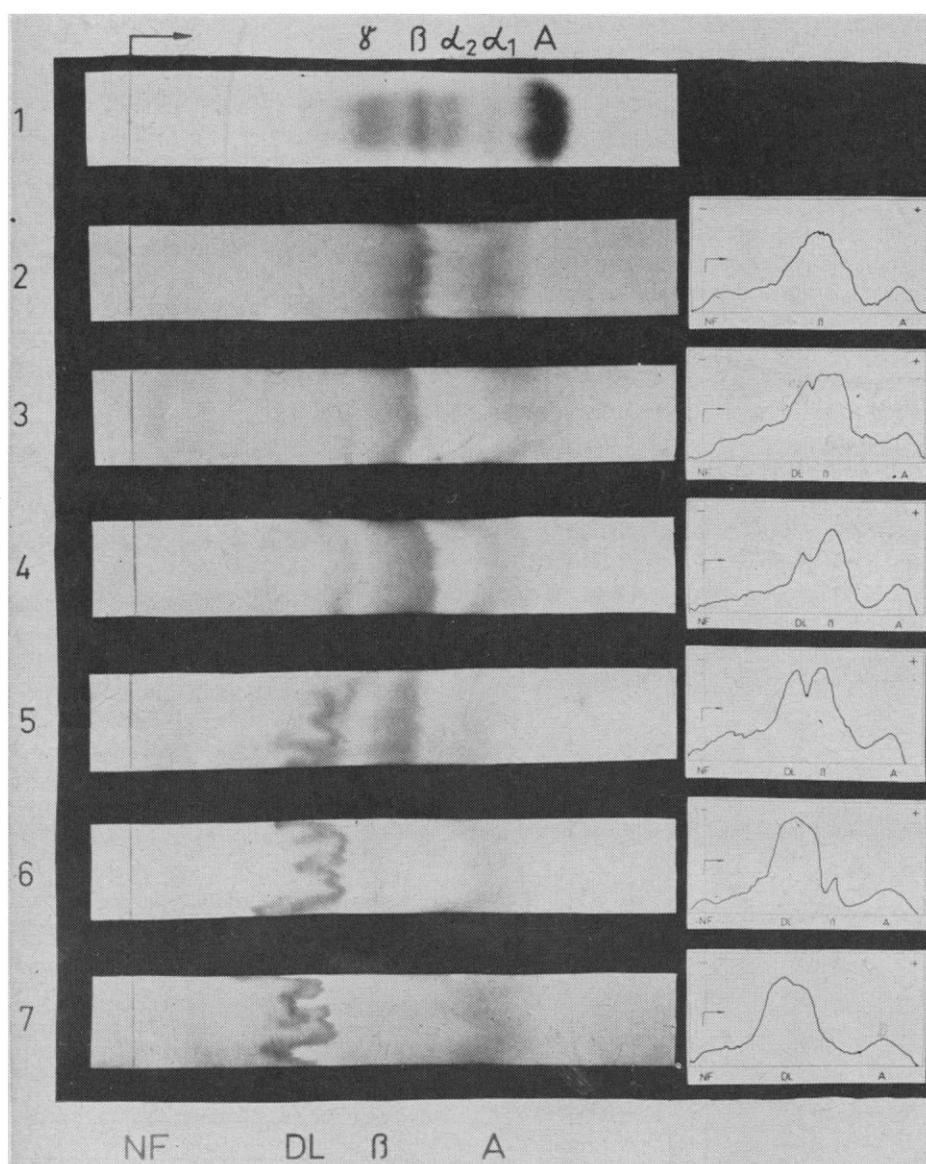


Fig. 1. Paper strip electrophoresis of human serum and mixtures of 1 ml of serum and 0.5 ml of a 6-percent colloidal solution of dextrans with increasing molecular weights, and densitometric scannings of the lipidograms. 1, Serum unmixed, protein staining. 2, Serum unmixed, lipid staining. 3-7, Mixtures of serum and dextran (Molecular weights of dextran: 3, 10,000; 4, 40,000; 5, 80,000; 6, 150,000; and 7, 500,000). Note the appearance of the dextran-lipid fraction (DL-fraction) on the lipidograms and densitometric scannings.