alanine and its hydroxylated derivatives is unknown.

These data suggest that a single administration of L-dopa causes transient but extensive changes in the mechanisms responsible for brain protein synthesis. Our data do not distinguish between possible effects on glia and on neurons. Since the neurons contain an abundance of free polysomes compared to membrane-attached polysomes, whereas the glial cells have more of the latter, it is possible that specific changes in neuronal polysomes would be indicated if the disaggregation is confined to the free polysomes. The unexpected increase in the amount of free tryptophan in brain that follows a single dose of L-dopa indicates that the mechanism by which L-dopa disaggregates brain polysomes probably differs from the mechanism of disaggregation by L-phenylalanine which is accompanied by a fall in free tryptophan levels (*12*). It is possible that L-dopa acts on polysomes by limiting the availability of other amino acids, for example methionine (*9*).

In studies to determine the mechanism for the increased brain tryptophan we have found thus far that administration of L-dopa (500 mg/kg) to 100- to 120-g rats results in a rise in plasma tryptophan associated with the rise in brain tryptophan. The increase in tryptophan in plasma after administration of L-dopa was proportionately greater than that in the brain, resulting in a decrease in the ratio of tryptophan in brain to that in plasma. The concurrent increase in tryptophan in plasma and brain is not surprising, because physiological variations in the tryptophan content of brain have been reported to be associated with the amount of tryptophan in plasma, as exemplified by their daily rhythms (*17*).

It is not known whether the changes in polysome profile reported here are associated with parallel changes in brain protein synthesis. Such changes could participate in either the therapeutic or the toxic actions of the drug.

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Polysome pellets were prepared by layering 6 ml of the postmitochondrial supernatant on a discontinuous sucrose gradient with 3 ml each of 2*M* sucrose in TKM buffer and 0.5*M* sucrose in TKM buffer. Gradients were spun in the L2 Spinco ultracentrifuge at 40,000 rev/min (105,000*g*) for 4 hours in a Ti50 rotor. Pellets were washed with 5 ml of cold TKM buffer, carefully suspended in 0.3 ml of the same buffer, and frozen at —40°C until they were used in 1 or 2 days.

The thawed ribosome preparations were incubated in a 37°C water bath for 2 minutes before they were applied to the linear 10 to 40 percent continuous sucrose gradients. The gradients, done in duplicate, were spun in an SW50 rotor at 38,000 rev/min for 70 minutes, in the L2 Spinco ultracentrifuge. The extinction profiles at 260 nm were recorded automatically with a Gilford model 2000 spectrophotometer, as the gradient was displaced upward through a flow cell.
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Identification of the Germination Self-Inhibitor from Wheat Stem Rust Uredospores

Abstract. *Two germination inhibitors from wheat rust uredospores were identified as the* cis *and* trans *isomers of methyl 4-hydroxy-3-methoxycinnamate (methyl ferulate). They are the self-inhibitors from these spores described previously.*

Rust fungi are among the most important disease agents of plants. In nature, these fungi are obligately dependent upon their hosts and are highly selective in regard to host compatibility. Uredospores of the rust fungi fail to germinate if floated in dense populations on water because endogenous self-inhibitors released from the spores prevent initiation of germ tubes (*1*). The ecological function of the self-inhibitors apparently is to minimize spore germination where survival would be poor, especially within the fructification structure.

Self-inhibitors of spore germination were first described for the rust fungi by Allen (*1*), when he found that crowding of wheat stem rust uredospores reduced their germination. Washing the spores with water reduced the self-inhibition, but the solutions on which the uredospores were floated contained a substance which was highly active in preventing germination. We report here the isolation and identification of this inhibitor.

Inhibitors were extracted from uredospores of the wheat stem rust fungus (*Puccinia graminis* Pers. var. *tritici* Eriks. & E. Henn., race 56) essentially as described previously (*2*). Uredospores were stirred in water (1 g/50 ml), and the inhibitor was re-

covered by filtration. The inhibitors were partitioned into diethyl ether and taken to near dryness on a rotary evaporator. The residue was then taken up in a small volume of ether, spotted onto preparative (0.5 mm) thin-layer plates of silica gel, and chromatographed in a solvent consisting of benzene and ether (80 : 20 by volume). After extraction from silica gel with ether, the preparation was dried and sublimed under vacuum in a short-path (20 mm) apparatus onto a condenser cooled with Dry Ice in acetone.

The chromatographic mobility (R_F) of the zone which contained the inhibitor was found in preliminary tests to be about 0.5 and coincided with an ultraviolet-fluorescent band about 1 cm wide at that R_F. To isolate the inhibitor without exposing it to ultraviolet light, we located the fluorescent zone by guide spots while the remainder of the plate was kept darkened. The silica gel containing the zone was scraped from the plate, extracted, and bioassayed. This zone contained as much inhibitor as the original ether solution applied to the plate, and tests of 1-cm bands of the plate showed very little activity elsewhere. The potency of the inhibitor was assayed at each step in the purification process by similar procedures.

The mass spectrum of a sample of the sublimate showed the following diagnostic peaks [mass to charge ratio (*m/e*); parent peak (P)]: 208 (P, base peak); 193 ($P - CH_3$); 177 ($P - OCH_3$); and 149 ($P - COOCH_3$). In addition, there were fragments at *m/e* 145, 117, 105, 91, and 77 indicative of aromaticity. The infrared spectrum had absorption peaks at 3430 cm^{-1} (hydroxyl), 1702 and 1718 cm^{-1} (ester carbonyl), 1598 and 1634 cm^{-1} (a pair of peaks of equal intensity), 1512 cm^{-1} (aromatic), and 1264 cm^{-1} (aryl ether). The ultraviolet absorption spectrum of the inhibitor dissolved in absolute methanol had absorption maximums at 325, 295, 235, and 217 nm. The spectra suggested that the inhibitor was the methyl ester of ferulic acid. The infrared and ultraviolet spectra of methyl isoferulate were not compatible with the spectra of the inhibitor.

The masses and relative abundance of the various fragments in the mass spectrum of synthetic methyl *trans*-ferulate (*3*) were identical to those of the unknown. The infrared spectrum of the inhibitor was consistent with that of a mixture of the *cis* and *trans* isomers of methyl ferulate. The *cis* isomer was prepared from the *trans* isomer by irradiation in methanol for 3 hours with ultraviolet light at 254 nm. The mixture of isomers was then resolved and purified by thin-layer chromatography on cellulose plates irrigated with water. Identity of the synthetic and natural *cis* and *trans* isomers was verified by infrared spectroscopy and by chromatography on thin-layer plates of cellulose (R_F 0.6 and 0.3, respectively). We conclude that the inhibitor from wheat rust uredospores is a mixture of methyl *cis*-ferulate and methyl *trans*-ferulate. Further chromatographic and infrared studies showed that a fresh sample of this inhibitor is composed predominantly of the *cis* isomer.

The extinction coefficient of a 1 percent solution of methyl *trans*-ferulate measured through a 1-cm light path at 325 nm was 750 in absolute methanol. With this value, it was determined that approximately 0.5 μg of native inhibitor can be extracted from 1 g of spores by our procedures. The ED_{50} of the unresolved mixture of isomers as determined from a plot of the probit of percent inhibition of germination against the logarithm of concentration was 8×10^{-9} mole/liter. The native inhibitor and the synthetic esters were equally toxic to spore germination, since their ED_{50} values were identical.

The self-inhibitors of uredospores of three other species of rust fungi—bean (*2*) and snapdragon and sunflower (*4*)—were each shown recently to be methyl 3,4-dimethoxycinnamate. Our finding that methyl ferulate is the self-inhibitor of wheat stem rust uredospores suggests that self-inhibitors in rust uredospores are a family of cinnamic acid derivatives.

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Pseudomonas aeruginosa: Growth in Distilled Water from Hospitals

Abstract. Pseudomonas aeruginosa *can grow relatively fast in distilled water obtained in hospitals and achieve high cell contaminations which remain stable for long periods of time. Cells grown in distilled water react quite differently to chemical and physical stresses than cells grown in standard laboratory culture media.*

Pseudomonas aeruginosa is recognized as a major causative agent of hospital-acquired infections. It is commonly associated with urinary tract infections and many generalized and subsequently fatal infections in infants and weakened patients. This organism can utilize a broad range of compounds as carbon and energy sources and may therefore survive and multiply in many liquids (*1*), including fuels (*2*). In 1962, Leifson detected this organism and several other Gram-negative bacterial species in 36 samples of distilled water from ten sources (*3*). When the organism is found in distilled water, however, it is often assumed that it is simply surviving or else growing very slowly. We found that *P. aeruginosa* will grow at a rapid rate in distilled water and that cells grown in distilled water react to various chemical stresses in a manner significantly different from that of cells grown in conventional laboratory culture media.

During a survey of environmental microbial contamination in a hospital pediatric ward, relatively high levels of Gram-negative bacteria, including *P. aeruginosa* and fecal coliform bacteria, were detected in the distilled water reservoirs of several mist therapy units.