number of stools to 1.6 a day and the wet weight to an average of 232.1 g a day. This represented a stool volume almost double the normal. The methyl cellulose stools had a slightly higher water content than the controls, and were semi-soft and easy of passage. No colicy or griping pains were experienced as a result of the drug administration.

The averages indicated that each gram of methyl cellulose ingested increased the bulk of the stools about 10 g. The greater part of this increase was water held by the colloid, although the dry weight increased somewhat more than could be accounted for by the dry weight of the methyl cellulose administered. Comparison of these results with those previously reported with gum karaya and psyllium[5] showed that gram for gram methyl cellulose had greater effect than these others. It would, therefore, seem to merit more extensive laboratory and clinical investigations.

The observations here reported are not enough in themselves to establish the clinical usefulness of this material. The toxicity studies carried on were not extensive enough to establish its safeness for repeated human consumption. For instance, the possibility of splitting off of the toxic methoxy groups in the body should be considered. It should be tested clinically in a variety of dysfunctions of the gastrointestinal tract to explore its general usefulness. Since it is not possible to continue these studies, the data obtained are presented in their present form for the value they may have to others interested in this problem.

Conclusions. 1. Methyl cellulose is a synthetic material having physical and chemical properties which would make it suitable for use as a colloid laxative. 2. Feeding it in a 10% concentration in the diet of rats, so they ingested an average of 11.4 g per kilo daily for 95 days, caused no change in the growth rate of the males, but decreased the growth of females about 14%, apparently as a result of decreased food intake. There were no significant pathological changes in the medicated animals observed at autopsy. 3. In doses of 10 g a day methyl cellulose approximately doubled the volume of the stools and increased their frequency in normal human subjects, a desirable clinical result sought by the use of colloidal laxatives. Each gram of methyl cellulose ingested increased the stools approximately 10 g. 4. Both the toxicological and clinical studies should be extended before a general therapeutic use of the drug as a laxative is approved.

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Action of Antibiotic Substances Upon *Ceratostomella ulmi.**

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The ability of certain antibiotic agents to exert selective fungistatic and even fungicidal properties has been definitely established. This is true particularly of gliotoxin,[1] actinomycin[2] and hemipyocyanin.[3] The purpose of this investigation was to determine the effect of various antibiotic substances upon *Ceratostomella ulmi*, the causative agent of the Dutch elm disease. This problem represents two significant aspects: (a) the economic importance of this organ-

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Soil Microbiology.

1 Weindling, R., and Emerson, O. H., *Phytopath.*, 1936, **26**, 1068; 1937, **27**, 1175.

2 Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1941, **42**, 231.

3 Stokes, J. L., Peck, R. L., and Woodward, C. R., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 126.

ism, and (b) its peculiar physiology, namely, its inability to synthesize vitamin B_6 or pyridoxine.[4]

The results of a study of the comparative effects of 7 typical antibiotic agents upon *C. ulmi* are illustrated in Table I. Two of the preparations proved to be highly effective, 2 were fairly active, one had only limited action, and 2 had no fungistatic activity at all, at least in the concentrations used. The 3 most active preparations were also strongly fungicidal.

TABLE I.
Fungistatic and Fungicidal Action of Antibiotic Substances upon *Ceratostomella ulmi*.

Nature of Substance	Mg of substance per 6 ml of nutrient broth		
	Fungistatic action		Fungicidal action in 48 hr
	Complete	Partial	
Penicillin	0	0	0
Actinomycin	0.1	0.03	0.1
Streptothricin	0	0	0
Clavacin	0.15	0.045	<0.15
Fumigacin	0	5.0	0
Hemipyocyanin	0.5	0.1	0.1
Gliotoxin	0.5	0.1	—

An attempt was made to throw some light upon the mechanism of the action of these substances upon the fungus. Robbins' medium in a slightly modified form† was used for the growth of the organism. Pyridoxine (100 μ mg per liter), peptone (5 g per liter) and malt (2.5 g per liter) were added as sources of vitamin B_6. Twenty-five ml portions of the medium were distributed in 125 ml flasks, sterilized and inoculated with the same volume of a uniform suspension of the organism grown on agar slants or in the above synthetic medium. Incubation took place at 28°-30°C for 4-7 days. Inhibition of growth was tested either by streaking agar plates or by diluting the culture and plating, using suitable agar media; the plates were examined and colonies counted after 2-4 days incubation. For measuring the total mass of growth, the combined cultures of duplicate flasks (total 50 ml) were filtered through Gooch crucibles, dried and weighed. The effect of two of the antibiotic substances found to be most effective in eliminating the growth of *C. ulmi*, namely, clavacin and actinomycin, is brought out in Table II. The nature of the medium greatly influences the extent of inhibition of the growth of the fungus by the antibiotic substances. The presence of peptone in the medium brought about reduced activity of the antibiotic substances upon the fungus. Whether this is due to the neutralization of the injurious action of the substance against a specific nutrient or function of the organism or whether the peptone served as an added nutrient remains to be determined.

In order to throw further light upon the effects of added nutrients in neutralizing the fungistatic activity of the two antibiotic substances, varying concentrations of pyridoxine and peptone were added to the above standard medium. Here as well, increasing amounts of pyridoxine or peptone were found (Table III) to neutralize partly the antibiotic effect of the two substances. The neutralizing effect was especially significant upon their fungicidal action. This was brought out in two different experiments. In one, a heavy suspension of *C. ulmi* cells was added to 25 cc portions of sterile water (Table IV) and in the other 3-day-old cultures of *C. ulmi* grown in the pyridoxine medium (Table V) were treated with the two substances. The viability of the *Ceratostomella* cells was determined by the streak method, and the actual numbers of living cells were determined by the regular plating procedure.

Actinomycin proved to possess lower fungicidal properties, as compared with its fungistatic action; clavacin is more highly fungicidal than actinomycin as compared with their respective fungistatic activities. In this respect, the fungicidal action of the two preparations is similar to their bactericidal action.[5] The presence of pyridoxine or pep-

[4] Robbins, W. J., and Ma, R., *Bull. Torrey Bot. Club*, 1942, **69**, 184.

† 50 glucose, 2 asparagine, 1.5 KH_2PO_4, 0.5 mg $SO_4 \cdot 7H_2O$, 0.02 $CuSO_4 \cdot 5H_2O$, 0.1 $FeSO_4 \cdot 7H_2O$ and 0.09 $ZnSO_4 \cdot 7H_2O$ in 1000 distilled water.

[5] Waksman, S. A., Horning, E. S., and Spencer, E. L., *J. Bact.*, 1943, **45**, 233; Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 609.

TABLE II.

Fungistatic Action of Clavacin and Actinomycin in Presence of Different Sources of B_6*

Source of B_6 in medium	Pyridoxine		Peptone		Malt	
Antibiotic agent, mg/ml	Viability†	Fungus growth,‡ mg	Viability	Fungus growth, mg	Viability	Fungus growth, mg
None	+++	23	+++	74	+++	101
Clavacin, 0.15	+++	16	+++	73	+++	110
" 0.6	+++	—	+++	68	+++	94
" 1.5	+	0	++	48	+	28
Actinomycin, 0.1	0	0	+	35	0	0

* Pyridoxine 100 μ mg per liter, peptone 5 g, and malt 2.5 g per liter.
† By streak method.
‡ Weight of dry fungus mycelium in 50 ml of medium, after 7 days incubation.

TABLE III.

Influence of Pyridoxine and Peptone upon the Fungistatic Activity of Antibiotic Substances. Original medium contained 100 μ mg pyridoxine per liter. Incubation 5 days.

Clavacin			Actinomycin		
Antibiotic substance mg in 25 ml	Pyridoxine, mg	Fungus growth†	Antibiotic substance mg in 25 ml	Pyridoxine, mg	Fungus growth
0	2.5	39	0	2.5	39
1.5	2.5	12	0.1	2.5	11
1.5	5.0	7	0.1	5.0	11
3.0	2.5	0	0.1	7.5	21
3.0	5.0	8			
3.0	7.5	22			
	Peptone, mg*			Peptone, mg*	
3.0	50	7	0.1	10	—
3.0	100	6	0.1	50	25
			0.1	100	21

* In addition to the 2.5 mg of pyridoxine in 25 cc of the original medium.
† Fungus growth reported on 50 ml basis.

TABLE IV.

Fungicidal Action of Clavacin and Actinomycin upon a Suspension of *C. ulmi* in Water.

Antibiotic substance		Peptone, mg	Viability		No. of fungus cells in 1 ml 4 days incubation
Nature	Amt added, mg		1 day	2 days	
0		0	+++	+++	2,800,000
Clavacin	1.5	0	++	0	0
"	1.5	50	+++	+	30,000
"	3.0	0	0	0	0
"	3.0	50	++	Tr	16,000
"	3.0	100	++	Tr	35,000
Actinomycin	0.1	0	+++	Tr	650,000
"	0.1	50	+++	+++	4,850,000
"	0	50	+++	+++	4,920,000

TABLE V.
Fungicidal Action of Clavacin and Actinomycin upon Growing Cultures of *C. ulmi* in Pyridoxine Media.*

Antibiotic substance			
Nature	Amt added,† mg	Additional pyridoxine, μ mg	*C. ulmi* cells in 1 ml
Actinomycin	0.1	0	2,570,000
"	0.1	5	7,750,000
Clavacin	1.5	0	1,000,000
"	1.5	2.5	1,200,000
"	1.5	5.0	1,500,000

* Materials added to 3-day-old cultures and plated after 2 days further incubation.
† To 25 ml of culture.

tone tends to overcome the activity of actinomycin to a greater extent than that of clavacin.

It is of interest to report here an unpublished observation,‡ made during a study of the effect of vitamins upon the antibacterial activity of actinomycin, that vitamin C was the only one that exerted a partial inhibiting effect upon this substance.

Summary. A study has been made of the fungistatic and fungicidal action of various antibiotic substances upon *Ceratostomella ulmi*, the causative agent of the Dutch elm disease. Actinomycin and clavacin had a strong effect upon this fungus, penicillin and streptothricin had no effect at all, and other substances fell between.

The antifungal activities of clavacin and especially of actinomycin could be partly overcome by certain nutrients in the medium, especially peptone. Whether this is due to the pyridoxine content, to some other neutralizing effect or to a nutrient effect of the peptone is still to be determined.

‡ This observation was made by Dr. H. Boyd Woodruff working in our laboratory.

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Protein Fractionation and Separation in the Large Tiselius Cell.*

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The Tiselius electrophoresis apparatus introduced to America about 1937 is now commonly found in the leading laboratories throughout the country. The War has focused much effort upon protein solutions, especially serum and plasma, and as a result the Tiselius apparatus found not only immediate but intense application.

In order to separate the fractions obtained by electrophoresis, Tiselius[1,2] has used 2 small so-called separation cells. A compensator mechanism made it possible to move mechanically the total protein solution until a desired fraction was concentrated within the upper or lower channel. One or both of the middle sections were slid out of alignment. The supernatant buffer was removed, and finally the fractions in the various compartments were obtained individually by means of an extended steel needle attached to a syringe. Longsworth[3] recently has improved the "convection-proof" pipet technic of Tiselius[4] by lowering a fine glass capillary into the cell and very carefully withdrawing a desired fraction with a fixed syringe-compensator device. The removal of any fraction could be accurately controlled by direct Schlieren observation.

The method of Tiselius is satisfactory but causes undue dilution of the desired component, while the Longsworth technic requires additional apparatus and extreme

* Aided by a grant from the Commonwealth Fund.

1 Tiselius, A., *Trans. Faraday Soc.*, 1937, **33**, 524.
2 Tiselius, A., *Biochem. J.*, 1937, **31**, 1464.
3 Longsworth, L. G., *Chem. Rev.*, 1942, **30**, 323.
4 Tiselius, A., *Kolloid-Z.*, 1938, **85**, 129.