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It is of interest to report here an unpublished observation,‡ made during a study of the effect of vitamins upon the antibacterial activity of actinomycin, that vitamin C was the only one that exerted a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

C. L. SAN CLEMENTE. (Introduced by E. E. Ecker.)
From the Institute of Pathology, Western Reserve University, and the University Hospitals, Cleveland, Ohio.

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.

care, if diffusion is to be avoided.

This note describes a method to separate fractions in the single, large middle section of the Tiselius cell without extra equipment nor danger of diffusion. For example, fresh serum was diluted 1:2.5 with buffer and dialyzed one day in 2 liters of the same buffer. Electrophoresis was carried out in the usual way until the main components were separated almost completely from each other over two-thirds of the length of the channel. The middle section was moved to one side. The glass plug on the side arm of the "open" electrode vessel was removed, and all the buffer above the cell and from part of the main vessel was siphoned off. The middle section was slid back in place again; no movement of solution occurred because of the "closed" electrode vessel. The 3-way stopcock on the side arm of the "closed" electrode vessel was turned to permit continuity between the vessel and compensator syringe. The compensator motor was started and the topmost component was pushed slowly into the empty side arm of the "open" electrode vessel. The progress of this movement was followed easily by direct observation on the ground glass plate. When this first component was just completely above the glass flanges of the cell, the motor was stopped and the middle section was moved out of alignment. A long needle on a syringe was used to remove the freed component. After rinsing the side arm, the middle section was realigned, and each of the components was separated in the same manner.

The advantages of the above technic include: (1) simplicity and rapidity; (2) elimination of special extra equipment; (3) avoidance of dilution beyond that which has occurred from the migration of the respective component; (4) minimizes the danger of diffusion between components. It is true that a small amount of overlapping occurs between fractions but this factor may be eliminated by combining the respective components from several runs of whole material, followed by electrophoresis in the same manner of each of the components separately. The small amount of faster and slower moving material traveling with the main component can now be "cut" off without much difficulty.

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Influence of Incubation at 37° C on Stability of Thiamin and Riboflavin in Cow's Milk.*

BARNETT SURE AND ZENAS W. FORD.

From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.

Our recent finding[1] that animal tissues destroy appreciable amounts of thiamin and riboflavin during incubation at 37°C for 24 to 48 hours suggested a study of the stability of these vitamins in cow's milk under similar treatment. Pasteurized cow's milk, obtained from the University Creamery, was used throughout this investigation. Similar results, however, were obtained with raw untreated milk.

The experiments were carried out with 20 cc samples of milk, which contained 4.95 to 7.7 μg thiamin and 29 to 32 μg riboflavin. In the first experiment, 4 20 cc samples of cow's milk, to which have been added 0 μg, 10 μg, 100 μg and 1,000 μg of thiamin and equal doses of riboflavin, were incubated at 37°C for 48 hours. It will be noted from Table I that there was considerable destruction of thiamin in cow's milk during this

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[1] Sure, B., and Ford, Z. W., *J. Nutr.*, 1943, in press.