hydrochloride, eserine sulfate, atropine sulfate, nicotine and sodium fluoride, in 100 γ quantities, and cysteine hydrochloride, potassium thiocyanate, potassium cyanide, and the sodium salt of heparin‡ in 1 mg quantities were also inactive.

Summary. Acetylcholine, epinephrine, a series of alkaloids, and several other substances were without influence on the hydrolysis of adenosine triphosphate. Copper and zinc salts inhibited the reaction.

‡ This was obtained from Hoffmann-La Roche and Co., Nutley, N.J.

13921 P

In vivo Activity of Streptothricin Against *Brucella abortus.**

H. J. METZGER, SELMAN A. WAKSMAN AND LEONORA H. PUGH.
From the New Jersey Agricultural Experiment Station, New Brunswick, N.J.

An antibiotic substance possessing rather strong bacteriostatic and bactericidal properties against many gram negative and certain gram positive bacteria has been isolated from a soil *Actinomyces* by Waksman and Woodruff[1] and designated as streptothricin. This substance has now been tested extensively against *Brucella abortus,* the causative agent of brucellosis, and was found to have a strong inhibitory action against this organism not only *in vitro,* but also *in vivo.*

Bactericidal tests of the action of streptothricin† were made by adding graduated doses of the crude preparation of this substance to 10 cc portions of a suspension of *Br. abortus* in nutrient broth and incubating 2 hr at 37°C. This incubated suspension was then plated on tryptose agar. No growth of the organism was obtained on plates inoculated from the preparations containing 0.1 mg or more of the crude streptothricin per 10 cc of broth.

The *in vivo* tests of the activity of streptothricin against *Br. abortus* were at first conducted with incubating eggs. Toxicity tests indicated that the chick embryo tolerated enough of the crude preparation of this substance to warrant testing its activity against this pathogen in such a living medium.

A series of groups of 15-day incubated eggs were inoculated on the chorioallantoic membrane with approximately 2,000 *Br. abortus* cells, using 48-hr-old cultures of Strain No. 19 grown on tryptose agar, suspended in saline solution. Cultures made from control groups of eggs 4 to 5 days after inoculation indicated this to be an infective dose. On the day following the inoculation of the eggs, those scheduled for treatment were given varying amounts of streptothricin, all of which were below the toxic level. The material was administered on the chorioallantoic membrane. These treated eggs were cultured on the 19th or 20th day of total incubation. The results showed that 10 mg of crude streptothricin, administered 24 hr after the inoculation of the eggs with *Br. abortus,* had been sufficient to bring about complete destruction of this organism in the living chick embryo.

These experiments with chick embryos were followed by other experiments employing guinea pigs. At first, an initial dose of 100 mg of crude streptothricin was administered simultaneously with the inoculation of the animal with a small infective dose of *Br. abortus.* The streptothricin was given intraperitoneally to one group and subcutaneously to another group of animals, whereas the controls were left untreated.

* Journal series paper, New Jersey Agricultural Experiment Station, Rutgers University, Departments of Dairy Husbandry and Soil Microbiology.

1 Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 207.

† We are indebted to Merck and Company, Rahway, N.J., for supplying some of the streptothricin used in this work.

Following the initial dose, this treatment was continued for 4 weeks at 5-day intervals, so that each guinea pig received a total of 600 mg of the crude streptothricin. After 4 weeks all 3 groups of animals were bled from the heart and killed for culturing. The blood serum of the treated groups showed no *Br. abortus* agglutinins in a 1:25 dilution, whereas that of the control group agglutinated completely in dilutions varying from 1:25 to 1:400. *Br. abortus* was recovered from all untreated controls but from none of the treated guinea pigs.

In another experiment, the treatment with streptothricin was started one week after the inoculation of the guinea pigs with approximately 80,000 *Br. abortus* cells. A total of 1200 mg of crude streptothricin was administered to each animal intraperitoneally at regular intervals over a period of 3 weeks, after which the treated and the control animals were examined as in the previous experiment. The blood serum of the treated animals showed no *Br. abortus* agglutinins in a 1:12.5 dilution, whereas that of the controls agglutinated completely in dilutions varying from 1:50 to 1:400. *Br. abortus* was recovered from the spleens of both the treated and the control groups but plate counts made from ground splenic pulp indicated that there was a much greater concentration of organisms in the spleens of the untreated controls than in the treated group.

Summary. Streptothricin, an antibiotic substance obtained from a soil *Actinomyces*, has been tested against *Brucella abortus* both *in vitro* and *in vivo* with favorable results. Experiments with incubating eggs established that the toxicity of streptothricin is low enough to make possible the administration of doses sufficient to destroy *Br. abortus* in the living tissues. Studies with guinea pigs indicated that streptothricin offers considerable promise as an antibiotic agent against brucellosis in animals.

13922 P

A New Method for Separation of the Basic Amino Acids from Protein Hydrolysates.*

RICHARD J. BLOCK.
Cooper Road, Scarsdale, N.Y.

Four methods have been described for the separation of the basic amino acids from the other products of protein hydrolysis. The oldest is by precipitation with phospho-24-tungstic acid according to Hausmann[1] and to Van Slyke.[2] A second is by electrolytical transport introduced by Ikeda and Suzuki[3] and developed by Foster and Schmidt,[4] Kuhn and Desnuelle,[5] and others.[6-8]

Whitehorn[9] showed that arginine, histidine, and lysine were exchanged for Na^+ ions on the synthetic zeolite, "Permutit." An application of this finding is Dubnoff's[10] use of "Permutit" to separate arginine from

* Encouragement in carrying out this project was received from the Food Branch, War Production Board.

1 Hausmann, W., *Z. physiol. Chem.*, 1899, **27**, 95.

2 Van Slyke, D. D., *J. Biol. Chem.*, 1911-12, **10**, 15.

3 Ikeda, K., and Suzuki, S., U. S. Patent No. 1,015,891, Jan. 30, 1912.

4 Foster, G. L., and Schmidt, C. L. A., PROC. SOC. EXP. BIOL. AND MED., 1921-22, **19**, 348; *J. Biol. Chem.*, 1923, **56**, 545; *J. Am. Chem. Soc.*, 1926, **48**, 1709.

5 Kuhn, R., and Desnuelle, P., *Ber. deut. chem. Gesel.*, 1937, **70**, 1907.

6 Albanese, A. A., *J. Biol. Chem.*, 1940, **134**, 467.

7 Csonka, F. A., *Cereal Chem.*, 1941, **18**, 523.

8 Gordon, A. H., Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, 1941, **35**, 1369.

9 Whitehorn, J. C., *J. Biol. Chem.*, 1923, **56**, 751.

10 Dubnoff, J. W., *J. Biol. Chem.*, 1941, **141**, 711.