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.

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

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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

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a protein hydrolysate for subsequent estimation by the Sakaguchi reaction. The necessity of removing the basic amino acids from the zeolites with strong neutral salt (NaCl, KCl) solutions, largely precludes this procedure as a preliminary step for their further use.

Recently Turba[11] has used chromatographic adsorption on activated fuller's earths (Filtrol-neutrol and Floridin XXF extra) to separate arginine, histidine, and lysine from each other and from the monoamino acids. M/6 KH_2PO_4 and a mixture of N H_2SO_4 and pyridine (5:1) are used as the eluting agents. No report has been seen on the application of this procedure to protein hydrolysates.

The introduction by Adams and Holmes[12] of two types of ion exchange resins, one capable of fixing mineral and strong organic acids and the other able to bind cations, opened the possibility of separating amino acids from the non-nitrogenous compounds in protein hydrolysates. The resins, Amberlites IR-1, IR-100 and IR-14 (Resinous Products and Chemical Company) have proven to be especially valuable for the separation of the basic amino acids from the other constituents of protein hydrolysates. A concentrate is thus obtained which is very suitable for the isolation and large scale preparation of arginine, histidine, and lysine.

The following experiment is representative: 11.7 g of commercial blood fibrin (N 16.0%) were hydrolyzed with 200 cc of 1:1 HCl for 20 hr. The excess acid was removed by concentration *in vacuo* and the residue was taken up in 500 cc of hot water, decolorized with 2 g of activated carbon, and filtered. The residue was thoroughly washed. The combined filtrates were concentrated to dryness. The residue was taken up in 800 cc of water and stirred with the acid binding resin, IR-4, until the reaction of the solution was approximately pH 6. The resin was removed and thoroughly washed. The amino acid solution (volume 1500 cc) was passed through a 10″ x ¾″ column of cation adsorption resin, IR-100, (hydrogen cycle) at the rate of 40 to 50 cc per min. The column was washed with water and the basic amino acids were removed by exchange with 7% HCl. The completion of the removal was followed by testing the effluent with phosphotungstic acid and by the Sakaguchi reaction. The effluent solution was concentrated to remove the excess HCl and diluted to 100 cc. Total nitrogen, 538 mg; nitrogen *not* precipitated by phosphotungstic acid, 30 mg. Tests for cystine, tyrosine, proline, and hydroxyproline were negative. The basic amino acids were isolated from a 50 cc aliquot of this solution according to the modified Kossel procedure. Histidine was precipitated with Ag_2SO_4 and $Ba(OH)_2$ at pH 7.4 and isolated as the nitranilate. Arginine was precipitated with Ag_2SO_4 and $Ba(OH)_2$ in strongly alkaline solution and isolated as the flavianate. 100 cc of alcohol were added to the filtrate from the arginine silver precipitate and the solution was concentrated at low temperature to remove all the ammonia. Lysine was isolated as the picrate without phosphotungstic acid precipitation. Yields: arginine, 6.4%; histidine, 2.0%; and lysine, 7.5%. Electrolytical transport purification[6] of a fibrin hydrolysate yielded 7.0% of arginine, 2.2% of histidine, and 6.0% of lysine, calculated on the basis of 16.0% of nitrogen in the protein.

Summary. A method is described for the separation of ammonia, arginine, histidine, and lysine from the other constituents of protein hydrolysates, based on the use of synthetic ion exchange resins. The procedure is especially valuable for the preparation of concentrates of the basic amino acids.

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