Actinomyces sp. P–12: The production of an antiviral factor by this Actinomyces is shown in Table 4. Only five of the eleven tests indicated promise. The organism grew slowly in culture media, and no culture filtrate less than 11 days old showed any activity whatsoever. These filtrates showed no antibacterial action, although on dextrose-asparagine agar medium the organisms inhibited, by a streak test method, *S. aureus* and *B. subtilis,* but not *E. coli* and *S. lutea.*

Although these results indicate the possibility of detecting antiviral or virus-inactivating substances produced by microorganisms, no claims can be made as to the practical promise even of the three organisms discussed above. Actinomycin A is known to be highly toxic. The other two organisms have not been sufficiently studied to warrant any further statements concerning their antiviral properties.

Even the *in vitro* activity of a substance does not necessarily indicate any therapeutic potentialities, since the substance must be non-toxic, should not be inhibited by body fluids and should be able to act where virus infection occurs, namely inside the living cell.

SUMMARY

One hundred and fifty organisms, comprising bacteria, fungi and actinomycetes, were isolated from straw-compost, manure, soil, drainage material and soil enriched with virus concentrates, and were tested for antiviral activity *in vitro.* Three of these organisms gave indications of possible inactivation of some of the fowl pox virus, and, in one case, of the laryngotracheitis virus. The active principle of one of these organisms was actinomycin A, an antibacterial substance known to be highly toxic to animals. The other two organisms were less extensively studied, and no claims regarding their antiviral potentialities can be made at present.

DORIS JONES
F. R. BEAUDETTE
WALTON B. GEIGER
SELMAN A. WAKSMAN

DEPARTMENTS OF MICROBIOLOGY AND POULTRY SCIENCE,
NEW JERSEY AGRICULTURAL EXPERIMENT STATION,
RUTGERS UNIVERSITY

CRYSTALLIZATION OF A TRYPSIN INHIBITOR FROM SOYBEAN[1]

THE presence of a protease inhibitor in soybean has been recently reported by Ham and Sandsted[2] and by Bowman.[3]

The present communication deals with the isolation from cold-processed defatted soybean meal of a crystalline protein which inhibits the proteolytic action of trypsin.

Unlike the crystalline inhibitor isolated from beef pancreas[4] the soybean inhibitor is precipitated when heated in 2.5 per cent. solution of trichloracetic acid, and it does not diffuse through collodion or Cellophane membranes. It contains about 16 per cent. nitrogen and less than 0.01 per cent. of phosphorus. It is a protein of the globulin type, soluble in dilute acid, alkali or salt solution. Its minimum solubility in water is in the range of pH 4.2 to 4.8.

The light absorption spectrum of a solution of the crystalline soybean inhibitor is that of a typical protein with a maximum absorption at 2,800 Å and a minimum at 2,520 Å. It is free of carbohydrate, as tested by the Molisch reagent on one per cent. solution of the protein.

The trypsin inhibitory activity per mg of the material is not altered on repeated crystallization. It has very little inhibiting power on the proteolytic or the milk-clotting activity of chymotrypsin.

METHOD OF ISOLATION

The method of isolation of the inhibitor from soybean meal[5] consists essentially of the following steps:

(1) Washing of the meal with 80 per cent. alcohol.

(2) Extraction in 0.25 N H_2SO_4 in the proportion of 5 liters of acid per 1,000 gm of meal.

(3) Adsorption of the inhibitor from the acid extract on bentonite and elution with 5 per cent. solution of pyridine in water.[6]

(4) Precipitation of the inhibitor in amorphous form by titration to pH 4.4 at 10° C. after removal of the pyridine by dialysis.

(5) Crystallization at pH 5.0 and 35° C. This step consists in suspending the amorphous precipitate in twice its weight of water, warming the suspension to about 40° C., and titrating it with 1 N NaOH to pH 5.0 (tested with methyl red by the drop method on a test plate). The amorphous precipitate gradually dissolves, while crystals in the form of fine short needles and thin hexagonal plates appear at

[1] This work was initiated at the suggestion of Major I. A. Mirsky in connection with his studies on streptococcal fibrinolysin (see SCIENCE, 100: 198, 1944).

[2] Wendell E. Ham and R. M. Sandstedt, *Jour. Biol. Chem.,* 154: 505, 1944.

[3] Donald E. Bowman, *Proc. Soc. Exp. Biol. and Med.,* 57: 139, 1944.

[4] M. Kunitz and John H. Northrop, *Jour. Gen. Physiol.,* 19: 991, 1936.

[5] Soybean meal, Nutrisoy XXX, in the form of flakes, supplied by the Archer-Daniels-Midland Co., Chicago, Ill., was used throughout this work. The use of this meal was kindly suggested by Dr. M. L. Anson.

[6] The bentonite procedure was suggested by the work of G. Alberton, W. H. Ward and H. L. Fevold on "Crystallization of Lysozyme from Egg White," *Jour. Biol. Chem.,* 157: 43, 1945.

the same time. The crystallization is complete within a few hours if the solution is kept at about 35° C.

(6) Recrystallization. This consists in re-precipitating the protein at pH 4.4 and 10° C. from a relatively dilute solution of the crystals in water and then proceeding as described in (5). The inhibitor can also be recrystallized from 20 per cent. alcohol at pH 5.0.

The details of the method of isolation are to be described in a future publication.

Further studies are being made on the mechanism of the trypsin-inhibiting action of the new crystalline protein and also on some of its physical and chemical properties.

M. KUNITZ

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, N. J.

ELECTROPHORETIC STUDIES IN RHEUMATIC FEVER[1]

RECENT summaries of available clinical and epidemiological knowledge concerning rheumatic fever[2, 3] and investigations of this disease[4] indicate that rheumatic fever may not be a specific infectious disease but may be the result of a response on the part of the host to external stimuli which is different from the response to the same stimuli of individuals who do not develop rheumatic fever. Attempts have been made to correlate the occurrence of rheumatic fever with the antibody response of the host to infection with the group A–β hemolytic streptococcus, and these have been recently summarized.[5]

In considering this matter in a broader fashion, it is known that the measurement of serum antibodies is one method of determining the reaction of the host to external stimuli. Since it is also known that serum antibodies are for the most part found in the gamma globulin fraction of human serum[6] it seemed desirable to determine whether the gamma globulin fraction of patients suffering from rheumatic fever was different from that of normal individuals.

Isolated reports of electrophoretic studies in rheumatic fever indicate abnormalities in the gamma globulin fraction. Longsworth, Shedlovsky and MacInnes[7] included three specimens from cases of rheumatic fever among those from a large number of diseases without specifying stage of activity, and reported the gamma-albumin ratio to be elevated in all three patients. Luetscher[8] performed a similar study on a number of diseases and reported that a specimen from each of two cases of acute rheumatic fever with rheumatic heart disease and congestive failure contained a greater than normal amount of gamma globulin expressed as grams per hundred milliliters of plasma. In addition he stated that a specimen from one "old case" of rheumatic heart disease showed a similar change but no quantitative result was reported for that case.

METHOD OF STUDY

Electrophoretic patterns were obtained from the various sera in the Tiselius apparatus by using the "Schlieren-scanning method of Longsworth."[9, 7] Before electrophoresis the sera were diluted 1 : 4 or 1 : 6 with buffer solution of pH 7.6 containing 0.15 M. NaCl and 0.02 M. sodium phosphate and dialyzed against large volumes of this buffer at + 2° C. Measurements were made by a planimeter of the areas under the various peaks in the photographic record for computing the relative amounts of the various components present, using the patterns for the descending boundaries. The total protein content of the sera was determined by micro-kjeldahl nitrogen analysis.

RESULTS OF THE STUDY

As indicated in Table 1, the ratios of gamma globulin to albumin, and gamma globulin to total protein in nine specimens of serum from eight patients in the acute stages of the disease are elevated above "normal"[10] without exception, although one of these, serum (line 8, table 1), is at the upper limit of normal. Electrophoretic analysis of the serum in three of the same patients during inactivity of the disease show continued elevation of these ratios at a lower level. Four specimens from three individuals not suffering

[1] From the Bureau of Laboratories, Department of Health, City of New York; the Lederle Laboratories, Pearl River, New York, and the Kings County Hospital, Brooklyn, New York. This article was accepted for publication on January 29.

[2] May G. Wilson, "Rheumatic Fever," New York: The Commonwealth Fund; London: Oxford University Press, 1940.

[3] John R. Paul, "The Epidemiology of Rheumatic Fever and Some of Its Public Health Aspects," New York: The American Heart Association, Second Edition, Metropolitan Life Insurance Company Press, 1942.

[4] M. G. Wilson, M. D. Schweitzer and R. Lubschez, *Jour. Pediat.*, 22: 468 and 581, 1943. A. R. Rich and J. E. Gregory, *Bull. Johns Hopkins Hosp.*, 73: 239 and 465, 1943. H. Selye, O. Sylvester, C. E. Hall and C. P. Leblond, *Jour. Am. Med. Asn.*, 124: 201, 1944. M. G. Wilson, *Jour. Am. Med. Asn.*, 124: 1188, 1944.

[5] J. R. Paul, *loc. cit.*, p. 24.

[6] E. J. Cohn, J. L. Oncley, L. E. Strong, W. L. Hughes, Jr. and S. H. Armstrong, Jr., *Jour. Clin. Invest.*, 23: 417, 1944. J. F. Enders, *Jour. Clin. Invest.*, 23: 510, 1944.

[7] L. G. Longsworth, T. Shedlovsky and D. A. MacInnes, *Jour. Exp. Med.*, 70: 399, 1939.

[8] J. A. Luetscher, Jr., *Jour. Clin. Invest.*, 19: 313, 1940.

[9] L. G. Longsworth, *Jour. Am. Chem. Soc.*, 61: 529, 1939.

[10] H. Svensson, *Kolloid Ztschr.*, 87: 181, 1939. J. A. Luetscher, Jr., *Jour. Clin. Invest.*, 20: 99, 1941. D. H. Moore and J. Lynn, *Jour. Biol. Chem.*, 141: 819, 1941. V. P. Dole, *Jour. Clin. Invest.*, 23: 708, 1944.