Oberlin College, spoke on "The Role of Science in Regional Resource Development"; Walton Hamilton, professor of constitutional law at the Yale Law School, on "Public Policy in Respect to Technology"; Dr. Walter Rautenstrauch, professor of industrial engineering at Columbia University, on "Science Opens the Door of Production," and Lieutenant Steuart Henderson Britt, U.S.N.R., on "Discovery and Development of Scientific Talent."

AT a dinner of the alumni held as part of the commencement week program of the Massachusetts Institute of Technology, a gift of $350,000 was presented to the institute by Alfred P. Sloan, Jr., chairman of the Board of the General Motors Corporation. A gift of $100,000 was also announced from Gerard Swope, former president of the General Electric Company, to endow a group of post-graduate fellowships in the fields of physics, electrical engineering and industrial management. Fellowships awarded under this fund will go to students who have pursued courses especially planned for the education of superior students, special consideration to be given to students from St. Louis, Mo., Highland Park, N. J., and New Brunswick, N. J., or to students who are employees of the General Electric Company or their children.

THE University of Pennsylvania has received a bequest of $155,000 from the estate of Dr. George E. De Schweinitz, ophthalmologist, which will be used to establish a fund to support the department of ophthalmology and a professorship that will be named "the William F. Norris and George E. De Schweinitz Professorship of Ophthalmology." Dr. Norris was the first professor of ophthalmology in the School of Medicine of the university.

DR. DUNCAN MACINNES, of the Rockefeller Institute for Medical Research, New York City, who had been appointed the first Sigma Xi lecturer-in-residence, gave a series of lectures at Cornell University during the week of May 14 under the auspices of the Cornell Chapter and of the local members of the American Chemical Society. The titles of the lectures were: "Transference Numbers and the Debye-Huckel Theory"; "The Effect of Centrifugal Force on the Potentials of Galvanic Cells"; and "The Study of Proteins by the Electrophoretic Method."

THE Johnson and Johnson Research Foundation has established an annual award at Northwestern University. This consists of a silver medal and an honorarium of $250 to be known as the Malcolm T. MacEachern Award in honor of Dr. MacEachern, associate director of the American College of Surgeons and director of the program in hospital administration at Northwestern University.

SPECIAL ARTICLES

A SEARCH FOR VIRUS-INACTIVATING SUBSTANCES AMONG MICROORGANISMS[1,2]

THE results of studies of the action of antibiotic agents against true viruses have been almost consistently negative. Robinson[3] reported that gramicidin, tyrocidine, tyrothricin, penicillin, streptothricin, citrinin and gliotoxin are inactive against the viruses of the PR8 strain of epidemic influenza and a strain of *Lymphogranuloma venereum;* on the other hand, actinomycin exerted a slight *in vitro* inactivation of the influenza virus. Penicillin was found to be ineffective for experimental infections of vaccinia BH, St. Louis encephalitis and equine encephalomyelitis.[4] Subtilin and other agents were useless against influenza A virus in white mice.[5] Clavacin, in spite of the original claims, proved to be of no value in the treatment of the common cold.[6] Penicillin and clavacin were recently found to be inactive against the virus of fowl pox,[7] and aspergillic acid had no effect upon the encephalomyelitis virus.[8]

As contrasted with these negative results, certain claims have been made that filtrates of various fungi and of an undescribed species of *Actinomyces* have a high virucidal activity, both *in vitro* and *in vivo,* against the Frances strain of neurotropic yellow fever virus in mice.[9] These claims have not been confirmed as yet.

In any attempt to isolate chemical agents active against viruses, one is faced with the fact that these substances act only in the animal body. Whereas the action of antibiotic substances against bacteria, actinomycetes and fungi can be investigated readily by employing various plate methods,[10] the study of antiviral activity of various agents is complicated by the need of employing animals, tissue culture or egg

[1] Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Departments of Microbiology and Poultry Husbandry.

[2] Supported by a grant made by the Commonwealth Fund of New York.

[3] H. J. Robinson, Thesis, Rutgers University, 1943.

[4] R. F. Parker and H. W. Dieffendorf, *Proc. Soc. Exp. Biol. and Med.,* 57: 351, 1944.

[5] A. P. Krueger, SCIENCE, 98: 348, 1943.

[6] B. H. Robbins, *Proc. Soc. Exp. Biol. and Med.,* 57: 215, 1944.

[7] J. M. Stansfield, A. E. Francis and C. H. Stuart-Harris, *Lancet,* 247: 370, 1944.

[8] S. E. Sulkin and A. Goth, *Proc. Soc. Exp. Biol. and Med.,* 58: 16, 1945.

[9] H. Linhares, *O. Hospital,* 26: 327, 1944.

[10] S. A. Waksman and H. B. Woodruff, *Jour. Bact.,* 40: 581, 1940.

embryo techniques, in order to determine the survival or inactivation of a virus. In the following investigations, the developing chick embryo was selected as the test animal because it has been shown to be very sensitive to infection with certain viruses[11] and because its defense mechanism does not develop until near the time of hatching.

By the use of the above method, considered to be sufficiently sensitive to detect even slight amounts of virus inactivation and rapid enough for testing fairly large numbers of organisms, a survey was made of 150 cultures of bacteria, actinomycetes and fungi. Only three of these organisms were found to have even a slight *in vitro* inactivating effect upon the virus of fowl pox.

Viruses and Chick Embryos: The following viruses were isolated by one of us and used in these investigations: Fowl pox FH—egg passage strain; laryngotracheitis—egg passage strain; chick bronchitis—egg passage strain.

These viruses were maintained by inoculation into the chorioallantoic membrane of 11- to 12-day-old embryonated eggs, and were stored in a freezing unit at −20° C.

Embryonated eggs from White Leghorn hens were supplied by the College Farm of Rutgers University.

Isolation of Organisms: Various natural materials served as sources for the isolation of organisms. Straw-compost, manure, field soil, material from a drain in a poultry post-mortem room, and a soil enriched over a period of seven months with concentrates of fowl pox virus were used. Samples of these materials were plated out in various dilutions on a glycerol-phosphate agar medium to which fowl pox concentrate had been added as the source of nitrogen. The virus concentrate used for the preparation of the plates and for the soil-enrichment experiments was obtained by applying the method of differential centrifugation, used by Parker and Rivers[12] in purifying vaccinia virus, to suspensions of virus prepared by grinding infected chorioallantoic membranes under sterile conditions with saline and sand. The process was found to remove over 90 per cent. of the extraneous nitrogen from the original fluid. Following incubation of the plates at 28° C, colonies of the organisms developing were transferred to nutrient agar slants.

Tests of Culture Filtrates: Culture filtrates of the various organisms were obtained as follows: The bacteria were grown in nutrient broth; the actinomycetes and fungi in nutrient and in synthetic media containing 0.2 per cent. agar; in the case of shake cultures, the agar was omitted. The bacterial filtrates were sterilized by passage through Berkefeld or Seitz filters, and the filtrates of the cultures of fungi and actinomycetes by heating at 70° C for ten minutes.

The test procedure consisted in exposing a suspension of fowl pox virus to the culture filtrates of the various organisms (usually 0.15 ml virus suspension and 4.85 ml of filtrate) at room temperature for at least five hours, and then inoculating 0.2 ml portions into 11- to 12-day-old chick embryos. Following further incubation of the eggs for six days, the embryos were harvested and the extent of infection on the chorioallantoic membrane of the test mixtures was compared to that of the controls. The amount of virus used was so regulated as to induce a countable number of lesions on the membrane. Thus, by taking averages of a number of lesions, any reduction in infection would indicate a possible *in vitro* inactivation of the virus.

The advantages of this procedure lie in the ability to grow test organisms under optimum conditions, to treat the filtrate in any desired manner and to regulate the amount of inoculum so that a slight inactivation is not masked by an overwhelming dose of virus.

Experimental Results: One hundred and forty-seven of the organisms tested had no action at all upon the virus; in some cases the infection seemed to be actually enhanced. Three organisms were found, however, to have a definite inactivating effect, although this was rather slight and seemed to be unpromising. The organisms were a strain of *Actinomyces antibioticus* (S-4) and *Trichoderma* sp., both isolated from the straw compost, and *Actinomyces* sp. isolated from a soil enriched with fowl pox virus concentrate.

A. antibioticus S-4: Repeated tests with various culture filtrates of this organism showed a definite reduction in the average number of macroscopic lesions due to fowl-pox infection, as is brought out in Table 1.

TABLE 1

EFFECT OF CULTURE FILTRATES OF A. ANTIBIOTICUS S-4 ON FOWL-POX VIRUS

Culture medium in which organism was grown	No. of eggs	Average number of lesions per membrane
Glucose-tryptone	10	4
Glycerol-phosphate + virus concentrate	4	7
Nutrient broth	7	9
Starch-tryptone	7	10
Control	5	26

Extracts obtained from the culture filtrates of this organism by treatment with ether were active in the *in vitro* tests against fowl pox and laryngotracheitis, but inactive against chick bronchitis. Because of the

[11] F. M. Burnet, E. V. Keogh and D. Lush, *Austral. Jour. Exp. Biol. and Med.*, 15: 227, 1937.

[12] R. F. Parker and T. M. Rivers, *Jour. Exp. Med.*, 62: 65, 1935.

high toxicity of actinomycin, the active principle produced by this organism, further studies of its *in vivo* activity were hardly justified (Tables 2, 3).

TABLE 2

EFFECTS OF ACTINOMYCIN ON SURVIVAL OF FOWL POX VIRUS*

Number of eggs	Actinomycin per embryo, gammas	Survivors after 6 days	Average no. lesions
8	4	6	10
10	8	9	5
8	16	6	6
6	40	2	4
5	200	0	..
16	0	8	15

* In all cases virus was exposed to a concentration of actinomycin which was five times greater than that finally inoculated into the eggs.

TABLE 3

EFFECTS OF ACTINOMYCIN OF THE SURVIVAL OF THE VIRUS OF LARYNGOTRACHEITIS

Number of eggs	Gammas per egg	Number of embryos alive after*		Type of infection
		3 days	5 days	
		3	5	
10	10	7	1	reduced size of lesions
10	20	6	1	reduced size and number of lesions
10	30	6	3 (2 dying)	lesions fewer, smaller and more discrete
10	0	8	4	full infection; pronounced opaque primary and large areas of confluent secondary lesions

* Increase in deaths of treated eggs was due to the toxicity of the actinomycin. Examination of these eggs at five days revealed that the embryos had died only a short time before; therefore both living and dead embryos were considered in the analysis.

It is of interest that this organism produced typical actinomycin A, which was readily isolated in crystalline form.[13] The product formed orange-red crystals melting at 250°. A mixed melting point with actinomycin A obtained from the original *A. antibioticus* 3435 showed no depression. Determination of antibacterial activity also indicated that the two substances were identical. No antiviral or antibacterial activity was present in the B-fraction or in the aqueous residue after ether extraction. This shows that the antiviral activity of the organism was confined to the actinomycin A produced by it.

A comparison was next made of the purified actinomycin A isolated from the S-4 and 3435 strains.[14] Both had an *in vitro* inactivating effect; preliminary tests tended to indicate that the product obtained from the S-4 strain produced less toxic effects, *i.e.*, less necrosis at the point of inoculation and fewer necrotic secondary lesions (lesions on the chorioallantois other than those formed at the site of inoculation).

No attempt was made to use the substance as a therapeutic agent against experimental fowl pox infections.

Trichoderma sp. 117–15: In eight out of eleven tests made with this organism grown on several culture media, a definite *in vitro* destruction of the fowl pox virus appeared to be indicated (Table 4). In one of the cases, the infection was completely wiped out (no lesions were visible); however, since the pH of the filtrate tested had fallen to 3.9, the possible effect of the acid reaction was indicated.

TABLE 4

IN VITRO EFFECTS OF CULTURE FILTRATES OF TRICHODERMA SP. AND ACTINOMYCES SP. UPON FOWL POX VIRUS

Medium	Days	Condition of growth	Final pH	Inactivation of virus†
		Trichoderma filtrate		
NA*	7	Static	7.9	–
NA	11	Static	7.9	+
GTA	3	Static	7.9	(?)
GTA	12	Static	..	+
GT	6	Shaken	3.9	complete
GT	7	Static	4.9	(?)
GT	11	Static	6.8	+
ST	11	Static	7.8	–
CS	5	Static	4.9	+
CS	7	Static	4.7	–
CS	11	Static	7.2	(?)
		Actinomyces filtrate		
NB	5	Shaken	7.8	–
NA	7	Static	7.8	–
NA	11	Static	7.2	+
NA	11	Static	..	(?)
GT	5	Shaken	4.3	–
GT	7	Static	4.3	–
GT	11	Static	4.5	(?)
GT	24	Static	..	(?)
ST	5	Shaken	7.2	–
ST	7	Static	7.9	–
ST	11	Static	6.6	+

* NA = nutrient agar (0.2%); NB = nutrient broth; GTA = glucose-tryptone agar; GT = glucose-tryptone broth; ST = starch tryptone; CS = corn steep brown sugar medium.
† (?) = questionable reduction of the number of lesions due to the virus; + = reduction; – = no reduction.

Tests of the survival of the virus in various buffer solutions from pH 3.0 to pH 10.0 indicated that the concentrates of the virus are stable between pH 4.4 and 8.0. Above and below these values they undergo a progressive inactivation. At pH 3.9, the infection is definitely decreased.

Trichoderma was found to produce a substance inhibiting the growth of *B. subtilis* and *S. aureus,* especially in filtrates obtained from peptone-dextrose media.

[13] S. A. Waksman and M. Tishler, *Jour. Biol. Chem.*, 142: 519, 1942.

[14] S. A. Waksman and H. B. Woodruff, *Jour. Bact.*, 42: 231, 1941.

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.

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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.