of different tomato varieties for lycopersicin activity at all stages of growth from the seed to maturity. For this purpose the mechanically expressed, sterilized juice or sterile aqueous extracts of the plant tissue are placed directly in the assay cylinder. Sufficient data have not been obtained as yet to justify definite conclusions regarding the relative amounts of lycopersicin present in the materials which have been assayed; but it may be concluded that: (a) of the tomato varieties tested, including Bonny Best (highly susceptible to Fusarium wilt), Rutgers and Marglobe (resistant), Pan America and Red Currant (highly resistant), all contain the inhibitor; (b) lycopersicin activity, while absent in the seed, appears in seedlings germinated in the dark and in the plant within 8 days after planting; (c) the concentration of lycopersicin varies somewhat with the age of the plant and considerably with the plant part assayed. Results of these investigations, as well as consideration of the relationship between lycopersicin and the Fusarium wilt of tomatoes and consideration of the specificity of lycopersicin will be reported elsewhere.

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ASPERGILLUS USTUS

THERE are now in the literature several reports of antibiotics active *in vitro* against *Mycobacterium tuberculosis*. They have been derived from culture filtrates of a variety of molds including *Aspergillus fumigatus*,[1,2,3] *Actinomyces griseus*[4,5] and one of the Penicillium group.[6] The present report will describe yet another.

Early in 1944, while systematically examining the antibiotic-producing properties of a number of fungi appearing as contaminants on routine culture plates, we came upon one which made its culture medium highly active against *M. tuberculosis*. It has subsequently been identified[7] as a strain of *Aspergillus ustus*, (Bain) Thom and Church, and examined more completely for its several properties.

The mold grows well on Czapek-Dox medium with 4 per cent. glucose and 0.1 per cent. Bacto-Yeast extract added. After 36 hours' culture there appears on the surface of the medium a thin veil-like growth which on the following day or two assumes a pale blue-green color. As the spores begin to develop the growth becomes more intensely green, and ultimately forms a brown wrinkled membrane. Frequently one may observe numerous clear yellow droplets on the surface of the culture. The temperature for the optimal production of the antibiotic substance appears to be 28° C., but the fungus will grow over a wide range, including 37° C. With the progressive growth of the culture there is a gradual increase in pH of the medium from an initial 5.8 to a final value between 8.0 and 8.4.

The substance that inhibits the growth of *M. tuberculosis* can first be demonstrated on the sixth day of culture and continues to increase to a maximum concentration at 14 to 16 days. It can be extracted from the medium by the use of various solvents, such as ether, chloroform, acetone, or by adsorption onto Norit, followed by elution with ether. Extraction with ether at pH 8.0 to 8.4 yields a light yellow amorphous residue which is insoluble in water but soluble in either 1 per cent. sodium carbonate solution or 95 per cent. alcohol. The potency of this ether-extracted residue on the tubercle bacillus was determined by preparing serial dilutions of the dry crude residue in Long's synthetic medium and then planting on this medium a thin surface growth, approximately 7 mm in diameter, of *M. tuberculosis*, Strain H37. When examined after an incubation period of thirty days at 37° C., the tests usually showed complete inhibition of growth in dilutions varying from 1:200,000 to 1:400,000, but the activity of this ether extracted residue varied with each batch of substance tested. The controls, prepared in the same manner but without the addition of the residue, showed a heavy growth covering the entire surface of the medium at the end of thirty days. It is interesting to note that similar experiments conducted with *Mycobacterium ranae* showed that the growth of this organism was inhibited to the same extent as that of

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[1] A. Vaudremer, *C. R. Soc. Biol.*, 73: 51, 1912; 74: 278 and 752, 1913.

[2] M. A. Soltys, *Nature*, 154: 550, 1944.

[3] Igor N. Asheshov and Frieda Strelitz, SCIENCE, 101: 120, 1945.

[4] S. A. Waksman, *Proc. S.N. Mayo Clinic*, 19: 537, 1944.

[5] S. A. Waksman and A. Scholz, *Ibid.*, 57: 244, 1944.

[6] D. K. Miller and A. C. Rekate, SCIENCE, 100: 172, 1944.

[7] A culture of the fungus was sent to Dr. Charles Thom, and we are grateful to him for the above classification.

the tubercle bacillus. Against *Staphylococcus* and *Streptococcus* it has only slight activity, and against *Escherichia coli* none at all.

The active agent, whatever its nature, is very stable, for it is not completely destroyed even when autoclaved at a pressure of fifteen pounds for fifteen minutes. The filtrates from cultures kept at 28° C. for three months still show activity, and samples of the residue from ether extraction kept at 8° C. for the same length of time lose none of their potency.

Preliminary tests on mice have indicated that the crude extract is relatively non-toxic. Between 6 and 8 mgms can be tolerated by a mouse.

The experiments thus far have shown that there is an additional fungus of the Aspergillus group from the culture filtrate of which a substance can be obtained that definitely inhibits the growth of *M. tuberculosis in vitro*. It seems desirable, before attempting to establish the value of the antibiotic substance in experimental tuberculosis to obtain it in a more pure form. Studies are in progress to this end.

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THE EFFECT OF CYSTEINE ON STREPTOMYCIN AND STREPTOTHRICIN

THE recent communication of Cavallito and Bailey[1] describing the complete or partial inactivation of a large number of antibiotics by cysteine prompted us to test the action of the latter on streptothricin and streptomycin concentrates.[2] It was found that streptomycin is inactivated by cysteine, whereas streptothricin is not. Streptomycin is also inactivated by 2-aminoethanethiol but not to any significant extent by thioglycollic acid. The inactivation experiments were carried out by adding neutral solutions of the organic sulfur compounds to known amounts of streptomycin or streptothricin dissolved in neutral phosphate buffer. After storing for several hours, the solutions were tested for antibiotic activity against *Bacillus subtilis* by the Oxford cup method.[3]

TABLE 1

EFFECT OF ORGANIC SULFUR COMPOUNDS ON ACTIVITY OF STREPTOMYCIN AND STREPTOTHRICIN SOLUTIONS

Concentration of organic sulfur compound (mg/ml)	Streptomycin assay (units/ml)	Streptothricin assay (units/ml)
Control (phosphate buffer)	49	52
Cysteine HCl		
0.13	39	..
0.25	29	..
0.50	4	42
1.00	0	..
2.50	..	52
5.00	..	54
2-Aminoethanethiol HCl		
0.50	12	40
2.50	4	57
5.00	0	59
Thioglycollic Acid		
0.50	31	41
2.50	39	40
3.00	26	30

[1] Cavallito and Bailey, SCIENCE, 100: 390, 1944.

[2] The activity of streptomycin concentrates varied from 80 units/mg to 600 units/mg; the activity of the streptothricin was 440 units/mg.

The difference in behavior of streptomycin and streptothricin toward cysteine is of interest and of particular significance in view of the microbiological similarity of the two substances.[4] With the use of cysteine one can not only differentiate the two antibiotics but estimate the relative amounts of each in mixtures of the two (Table 2).

TABLE 2

EFFECT OF CYSTEINE ON MIXTURES OF STREPTOMYCIN AND STREPTOTHRICIN

	Streptomycin added (units/ml)	Streptothricin added (units/ml)	Cysteine hydrochloride added (mg/ml)	Assayed activity (units/ml)
Solution I ...	25	25	0	45
Solution II ..	100	0	2	0
Solution III .	100	8	2	9
Solution IV .	100	15	2	17
Solution V ..	50	50	1.3	45

The cysteine inactivation of streptomycin can be reversed by iodine; presumably cystine is formed during this process. To our knowledge, this is the first recorded instance of reversible cysteine inactivation of an antibiotic. The regeneration of the antibiotic activity of streptomycin solutions containing cysteine was carried out by shaking such solutions with small amounts of a carbon tetrachloride solution of iodine until no further decolorization occurred. The solutions were aerated to remove the organic solvent before assay. The recovery of activity was quantitative.

The observations thus far made indicate that the inactivation of streptomycin is reversible, not a property of the sulfhydryl group alone, nor is it limited to cysteine. A mechanism postulating either a reversible chemical reaction between the two substances or a competitive effect on metabolic processes would be consistent with these observations.

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THE MECHANISM OF PAIN IN TRIGEMINAL NEURALGIA[1]

TRIGEMINAL neuralgia (tic douloureux), an episodic, recurrent, unilateral pain syndrome, occurs for the