Table 1. Oxidation of reduced sulfur compounds by cell-free preparations of *T. thiooxidans*.

Substrate	Concn. (μM)	O_2 consumed (μmole)	SO_4 ion produced (μmole)
Na_2S	10	17.2	
$Na_2S_2O_3$*	10	13.0	10.5
$K_2S_4O_6$*	5	13.8	16.5
$K_2S_3O_6$	5	10.6	15.0
Na_2SO_3	10	3.5	10.4

* Reactions stopped before the oxidation was complete.

A bacterium corresponding to the original description of *T. thiooxidans* by Waksman and Joffe (*4*) was isolated from a sewage effluent at a spot where sulfur was deposited, by means of enrichment cultures in a mineral salts medium containing sulfur. The isolate is an obligatorily autotrophic, motile rod, 2 to 3 μ by 0.5 μ; it can oxidize sulfur to sulfuric acid at a *p*H less than 1.

Mass cultures were grown in the medium of the Baalsruds (*5*), supplemented with $FeCl_3$ (2 mg per liter). Frozen cell pastes were processed through a Hughes press, or cell suspensions in 0.1*M* phosphate buffer, *p*H 6.9, were disrupted in an 8-kcy/sec Raytheon supersonic oscillator. Cell-free extracts were prepared by centrifugation at 10,000*g* for 1 hour at 10°C.

The cell-free preparations rapidly and completely oxidized sulfide, thiosulfate, tetra- and trithionate to sulfate in the presence of O_2 (Table 1). That they contained cytochrome was shown by spectrophotometric examination of extracts reduced with dithionite (Fig. 1), which revealed absorption bands at 551, 522 and 420 mμ, corresponding to the α, β and γ bands of reduced cytochrome *c*, respectively. The same bands were observed if, instead of dithionite, any one of the above mentioned sulfur compounds was added to the extracts. This unequivocally demonstrates that the oxidation of substrates by *T. thiooxidans* can be coupled with the reduction of cytochrome, and thus supports the inference that the organism can derive its energy from a cytochrome-linked phosphorylation.

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Phytotoxic Substance from a Species of Penicillium

Abstract. *A* Penicillium urticae *Bainer, isolated from subsurface-tilled plots showing reduced wheat growth, produced a phytotoxic substance. The melting point and infrared and ultraviolet spectra of the crystalline material showed this substance to be patulin. Wheat shoot growth inhibition of 50 percent required 20, 20, and 75 parts per million of patulin in solution, sand, and soil culture, respectively.*

Subsurface tillage is a farming method to keep stubble mulch on the soil surface to control water and wind erosion. During a 23-year period at Lincoln, Nebraska, subsurface-tilled, as compared with plowed plots, on a corn, oats, and wheat rotation have produced, in the years of normal to above-normal rainfall, decreased yields and abnormal appearance of crops (*1*).

Fungi, isolated from subsurface-tilled soil showing reduced plant growth, were grown in potato dextrose broth at 28°C. Corn seeds were soaked in this liquid for 6 hours and placed in petri dishes between double layers of germination paper moistened with the liquid. The corn seeds were measured for percent of germination and growth lengths of shoot and root after germinating 3 days at 25°C. In a group of 91 isolates, 14 reduced germination to 50 percent or less.

One fungus, isolated from plots at Alliance, Nebraska, was identified as *Penicillium urticae* Bainer by the Northern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, U.S. Department of Agriculture, Peoria, Ill. This fungus, when grown in potato dextrose broth, produced a toxic material which caused severe stunting of germinating corn.

To obtain concentrated toxic material, the isolate was grown in 200 ml of potato dextrose broth in 1-liter erlenmeyer flasks for 14 days. The filtered culture fluid was flash-evaporated at 40°C and 2 cm-Hg pressure to 1/20 of its volume and extracted with ethyl acetate. Bioassay tests of the culture medium, culture fluid, condensed culture fluid, and ethyl acetate extract revealed the toxic principle to be generated by the fungus and to be readily extracted from the condensed culture fluid. The ethyl acetate extract was dried over anhydrous $MgSO_4$ and evaporated in a flash evaporator to a heavy, brown syrup which crystallized after standing for a few minutes.

The crude crystals were dissolved in ethyl ether and the solution was filtered to separate a small amount of dark-brown, insoluble material. Slow evaporation or chilling of the saturated ether solution produced colorless crystals with a melting point of 109° to 111°C uncorrected. In one experiment, 9 liters of original culture medium yielded 15.1 g of crystals.

Paper chromatography, using a developer of ethanol and water (4 : 1), separated the ethyl acetate extract into several components. Some of the components were fluorescent under an ultraviolet lamp with maximum intensity near 3600 Å. A complete chromatogram, cut into 1-cm sections, was tested for toxicity by placing separate sections between double layers of germination paper moistened with 6 ml of distilled water in 9-cm petri dishes. Five wheat seeds were germinated directly on each chromatogram section for 3 days at 25°C. A control consisted of a similar chromatogram of an ethyl acetate extract of the sterile potato dextrose broth. Only the chromatogram section with an R_F value of 0.76 proved to be toxic to the germinating wheat seeds. Chromatographing the crystalline material showed it to be toxic and to have an R_F value of 0.76. The physiologically active section of the chromatograms reacted with ammonia vapor at room temperature to give a visible, pale yellow spot fluorescing a bright tan color under the ultraviolet lamp.

Comparison of the melting point and infrared and ultraviolet spectra of the crystalline material with the corresponding characteristics of compounds known to be produced by *Penicillium urticae* Bainer showed the substance to be patulin (*2*).

Cheyenne wheat in bioassay tests was used to compare the effects of patulin on germinating seeds with 2,4-dichloro-

Table 1. The amounts of patulin, on oven-dry soil basis, required in autoclaved and non-autoclaved media to limit shoot length and weight of Cheyenne wheat seedlings to 50 percent of that of the untreated.

Treatment	Patulin concentration (ppm) required to limit	
	Shoot length	Shoot weight
Sand		
Autoclaved	13	20
Not autoclaved	32	50
Peorian loess		
Autoclaved	37	50
Not autoclaved	120	125
Sharpsburg silty clay loam		
Autoclaved	62	75
Not autoclaved	500	500

phenoxyacetic acid (2,4-D), indoleacetic acid (IAA), and coumarin. With a test substance concentration of 50 parts per million (ppm), germination as compared with untreated seed was as follows: 2,4-D, 40; coumarin, 80; IAA, 85; and patulin, 85 percent. Root growth was reduced to 50 percent of the untreated length by these concentrations: 2,4-D, 1 ppm; coumarin, 9 ppm; IAA, 25 ppm; and patulin, 20 ppm. Shoot growth was reduced to 50 percent of the untreated length by these concentrations: IAA, 63 ppm; coumarin, 20 ppm; patulin, 40 ppm; and 2,4-D, 7.5 ppm.

A test of patulin with Cheyenne wheat coleoptile sections, with and in the absence of small amounts of IAA, revealed that patulin acts as an auxin synergist at concentrations ranging from 0.01 to 10 ppm. Patulin is an unsaturated lactone and its synergistic effect is similar to that of coumarin. Patulin alone at low concentrations shows no growth-promoting effect on wheat coleoptile sections, and at concentrations exceeding 10 ppm it is inhibitory and toxic.

The effect of patulin on Cheyenne wheat seedlings grown in soil autoclaved for 1 hour at 15 lb/in.^{2} steam pressure and in nonsterile soil was tested with sand, Peorian loess, and Sharpsburg silty clay loam. Soil was placed in 9-cm petri dishes supported on No. 8 rubber stoppers, over water in 14-cm petri dishes in which were inverted 1-liter beakers so as to maintain a humid atmosphere. Duplicate dishes containing 15 seeds each were incubated for 5 days at a soil moisture level near saturation and at a temperature averaging about 25°C. After growth for 5 days, the shoots were cut off at the soil surface, measured for length, dried at 65°C, and weighed. The Sharpsburg soil, containing considerable organic matter and, hence, having the greater microbial activity, required larger amounts of patulin to reduce the growth of the plants (Table 1) than Peorian loess, which is low in organic matter. These results are in agreement with those of other investigators (*3*).

Adding patulin to the soil in amounts necessary to suppress plant growth to about 50 percent of normal caused a rapid development of a new microbial population. Many colonies of mold appeared on the surface of the Sharpsburg soil. Isolates of these colonies grown in potato dextrose broth developed substances toxic to corn seedlings. Microscopic examination showed the isolates did not belong to the *Penicillia*. Thus, although soils with the larger amounts of organic matter have a greater ability to neutralize the phytotoxic effect of patulin, they may have the potential to develop altered microbial populations that may produce substances strongly toxic to germinating seeds.

The patulin treatments in autoclaved and nonsterile soil showed root growth inhibition but not root curling and twisting that was observed with tests of the culture fluid and in the tests when *Penicillium urticae* Bainer was inoculated into sterile amended soil. This organism evidently produces another substance besides patulin that has an effect on root growth and causes root curling.

Patulin is produced by a number of fungi. Its effect on other fungi and, hence, indirect effect on crop plants may be significant. Stubble-mulch farming systems may provide ecological conditions such as concentration of organic matter and environmental relationships, especially in years of normal to above-normal rainfall, favorable for the growth of microorganisms producing phytotoxic substances.

Patulin production under field conditions and subsequent effect on field crops remain to be ascertained (*4*).

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Schistosoma mansoni: Development of Challenge Infections in Mice Exposed to Irradiated Cercariae

Abstract. *The principal effect of x-irradiated* Schistosoma mansoni *cercariae may be to slow the migration and development of the worms of a challenge infection. This would account for the smaller number of worms found in the portal system in the early weeks and the delayed accumulation of recoverable worms and eggs.*

When mice, rats or rhesus monkeys are exposed to irradiated cercariae of *Schistosoma mansoni* or *Schistosoma japonicum* and are later challenged with normal cercariae of the same species, they have fewer worms in the portal system, or fewer eggs in the feces or liver in the first 28 to 63 days (*1–3*). It has been concluded from these studies that the animals develop immunity as a result of aborted infections with the irradiated schistosomes. Generally, the difference in worm counts between "protected" and "unprotected" animals has been of the order of about 1 to 2, and Smithers (*2*) has emphasized the incompleteness of the protection afforded. However, in experiments with mice, in which the interval between challenge and examination was only 28 days, larger differences were seen.

Our experiments have produced additional information concerning the manner in which the changes induced by the irradiated cercariae affect the challenge infection.

Schistosoma mansoni obtained from Puerto Rico and maintained in a strain of snails from the same endemic area were used in female white Swiss mice that weighed 17 to 22 g at the start of the experiments. The mice were all ex-