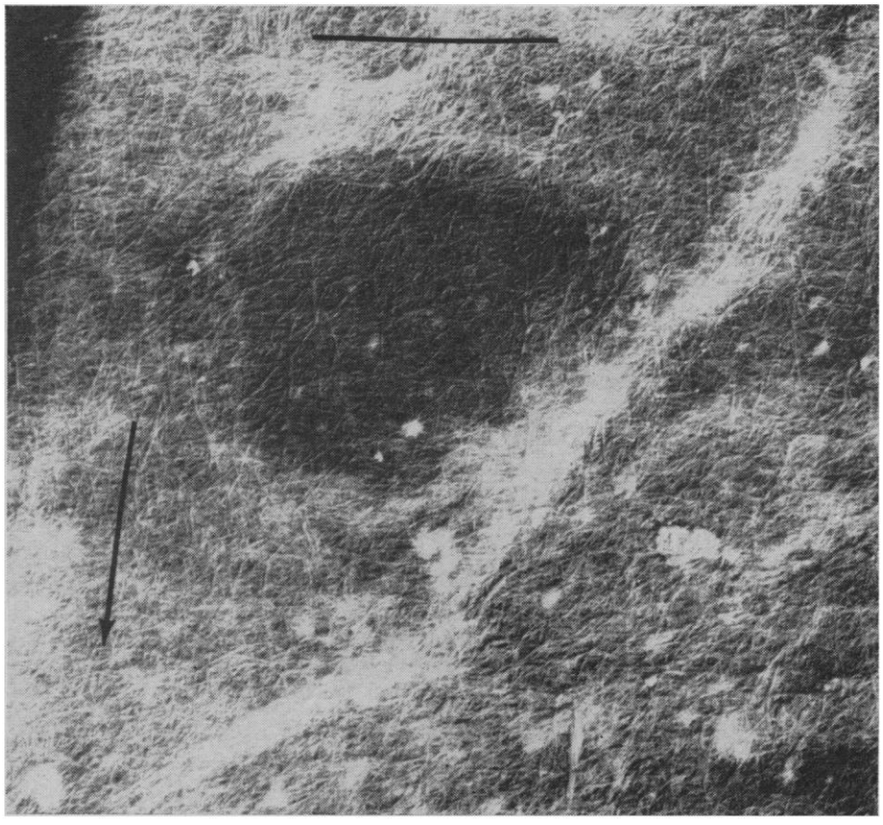

Fig. 2. Portion of a filament of *Helminthocladia californica*. The microfibrillar pattern of the cell wall and the pit base, as a whole, are reticulate. The arrow indicates the axis of the filament. Scale, 2μ.

length by apical growth. Protoplasmic connections are visible where the septa of the filaments are in contact. Other than these primary pit connections, no pitting is visible under the light microscope (*6*). Preliminary microchemical tests indicate the presence of pectic substances throughout the entire thallus. The reaction for cellulose is positive, and cellulose occurs both in the cell wall and in the mucilaginous sheath which surrounds the filaments.

Under the electron microscope, the reticulate microfibrillar pattern of the cell wall is clearly evident both in chemically cleared and in fresh material (Fig. 2). Although the filaments increase in length by apical cell growth, no difference in microfibrillar pattern or pitting has been observed between the older filaments of the medulla and the younger cells of the cortex. In addition to the pit connections of the perforate septa, termed primary pits by Fritsch (*6*), a second type of pitting is found on the radial walls where two filaments are in contact (Fig. 2). In these pit areas, actually thin areas in the loosely woven microfibrillar layer, the microfibrils are not masked by amorphous material.

In conclusion, the cell walls of brown and red algae examined consist of microfibrils with a diameter range of 100 to 250 angstroms. So far as the present survey indicates, a high degree of uniformity of microfibrillar orientation and pitting exists throughout the brown algae as represented by *Dictyota* and the red algae as represented by *Helminthocladia*. The two types differ as to the orientation of the microfibrils and the type and distribution of pitting (*9*).

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9. The assistance and guidance of Dr. Karl C. Hamner and Miss Virginia Sjaholm is gratefully acknowledged.

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Effect of Synthetic Polylysine on Fungi

Abstract. The synthetic, basic poly-α-amino acid, polylysine, had antifungal activity against plant pathogens (three strains of fusaria, three isolates of verticillia, and *Ceratocystis fimbriata*) and against the human pathogens (*Trichophyton mentagrophytes, T. rubrum*, and *Candida albicans*) in vitro. It inhibited penetration of *Ceratocystis fimbriata* on sweet potato slices. Polylysine inhibited the infection of tomato cuttings by *Fusarium oxysporum* f. *lycopersici*, but it was also toxic to the plants.

Several types of infective agents are inhibited by the synthetic basic poly-α-amino acid of lysine. Polylysine reduced the infectivity of tobacco mosaic virus; protected chick embryos against infection with animal viruses such as mumps, infectious bronchitis, Newcastle disease virus, and influenza B virus; and inhibited multiplication of bacteriophage (*1*). More recently polylysine has been shown to exert an antibacterial effect against certain bacteria both in vitro and in vivo (*2*), and to increase survival in mice bearing certain ascites tumors (*3*). This paper reports the effect of polylysine on the growth and invasiveness of certain fungi pathogenic to plants or human beings.

Polylysine was prepared by an ammonia-initiated polymerization of ϵ-carbobenzoxy-L-lysine N-carboxy anhydride in dioxane, in which the molar ratio of anhydride to ammonia was 20:1 (*4*). The polylysine was added to Czapek's salt solution (*5*), which contained 51 g of glucose per liter, to give a final concentration of polylysine ranging from 1 to 100 μg/ml. The flasks were inoculated with a heavy inoculum of spores; those inoculated with fusaria were incubated at 28°C; those with verticillia, at 21°C. A semisynthetic medium (*6*) was used for the experiments with *Ceratocystis fimbriata* Ell. and Halst. and was incubated at 28°C.

Polylysine (100 μg/ml of medium) inhibited the growth of *Fusarium oxysporum* f. *conglutinans* (Wr.) Synd. and Hans., *F. oxysporum* f. *cubense* (E.F.S.) Synd. and Hans., and *F. oxysporum* f. *lycopersici* (Sacc.) Synd. and Hans. for 2½ weeks; of *Verticillium albo-atrum* Reinke and Berth. isolate 4 (T-16) (*7*) for 3 weeks; and of *V. albo-atrum* isolate 50 for 2 months.

Verticillium albo-atrum isolate 1 and

Ceratocystis fimbriata still had no growth after more than 2 months. Material from flasks which had no visible growth a week after the original inoculation was reinoculated into media without polylysine. Only the *F. oxysporum* f. *cubense* and *F. o.* f. *lycopersici* then grew.

A synthetic poly-α-amino acid of glutamic acid containing approximately 20 glutamic acid residues was also tested for antifungal activity. This polymer actually appeared to stimulate growth of some of the fungi and to alter the growth characteristics of some slightly, but there was no strong inhibition of growth as with polylysine.

The effect of polylysine on the infection of sweet potatoes by *Ceratocystis fimbriata* was studied by taking slices from potatoes that had been sterilized by dipping in a 10-percent Clorox solution and streaking the cut surface with a spore suspension. Pieces of filter paper containing 0 to 10 mg of polylysine were placed on the center of the surface. The susceptible, Orange Little sweet potatoes which had been treated with polylysine appeared to have the same amount of fungal growth as the controls, but there was less penetration of the fungus into the sweet potato and less polyphenol formation. Treated resistant potatoes (Sunnyside) appeared to have less fungal growth, less penetration, and more callus formation under the paper than the controls. Neither the treated roots nor the controls showed appreciable polyphenol formation.

Polylysine was toxic to tomato plants and caused chlorosis, loss of lower leaves, stunting, immediate wilting, inhibition of root formation, and inhibition of transpiration at levels of polylysine higher than about 100 μg/ml (Fig. 1). The action of polylysine on the infection of tomato plants by *Fusarium oxysporum* f. *lycopersici* was also tested. Cuttings of 8-week-old Bonny Best tomato plants were placed for 24 hours in 25 ml of a spore suspension containing over 200,000 spores per milliliter, then transferred for 72 hours to Hoagland's solution (*8*) containing 0, 1, 10, 50, 100, or 500 μg of polylysine per milliliter, and finally transferred to Hoagland's solution alone. Polylysine did reduce infection by the fungus. After 2½ weeks three control plants were dead and one was only slightly green, whereas two out of four plants treated with 50 μg of polylysine per milliliter and all of those treated with 100 and 500 μg of polylysine per milliliter were alive (Fig. 2).

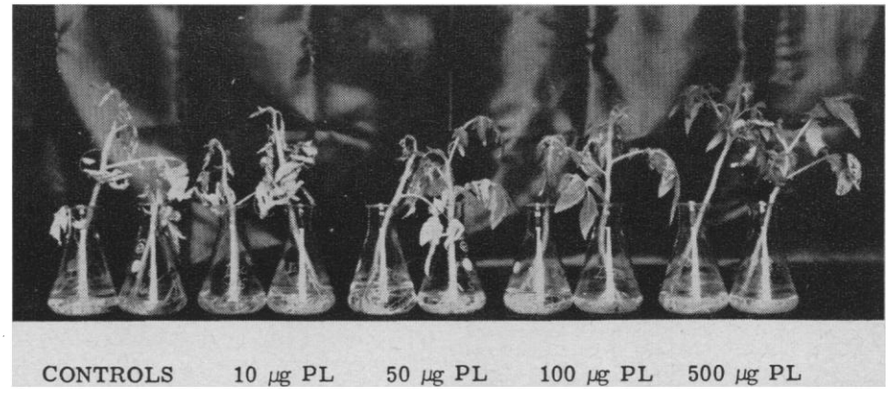

Fig. 2. Effect of increasing amounts of polylysine on the inhibition of *Fusarium oxysporum* f. *lycopersici* infection in tomato cuttings. Duplicate cuttings were photographed 2½ weeks after treatment with 0, 10, 50, 100, or 500 μg polylysine per milliliter of Hoagland's solution.

Polylysine was also found to inhibit spore germination and vegetative growth of the human pathogenic fungi *Trichophyton mentagrophytes* and *T. rubrum* at levels of 100 μg/ml of Difco Sabouraud dextrose agar medium and to cause about 50-percent inhibition of germination and growth of *Candida albicans* at that level. Polyglutamic acid had no effect (*9*).

It can be concluded that the synthetic, basic poly-α-amino acid, polylysine, had antifungal activity against the plant pathogens *Fusarium oxysporum* f. *conglutinans, F. o.* f. *cubense,* and *F. o.* f. *lycopersici, Verticillium albo-atrum* isolates 4 (T-16), 50, and 1, and *Ceratocystis fimbriata,* and against the human pathogens *Trichophyton mentagrophytes, T. rubrum,* and *Candida albicans,* in vitro. Polylysine inhibited fungal penetration and polyphenol formation in susceptible sweet potatoes infected with *Ceratocystis fimbriata* and caused some inhibition of fungal growth, a lessening of penetration by the fungus, and more callus formation in resistant sweet potatoes. Infection of tomato plants by *F. oxysporum* f. *lycopersici* was inhibited by polylysine, but polylysine was also toxic to the plants. Polyglutamic acid had no such effect on the fungi in vitro.

Polylysine would be very useful as an antifungal agent for in vitro experiments, especially where it is desirable to have an inhibitor with a simple structure, composed of subunits which are physiological in nature. However, its toxic effects on tomato plants suggests that it would not be a practical inhibitor for in vivo work on plants, and its slight inhibitory effect on fungus growth on sweet potato slices indicates that it would probably not be effective for sterilizing the surface of tubers, seeds, or bulbs (*10*).

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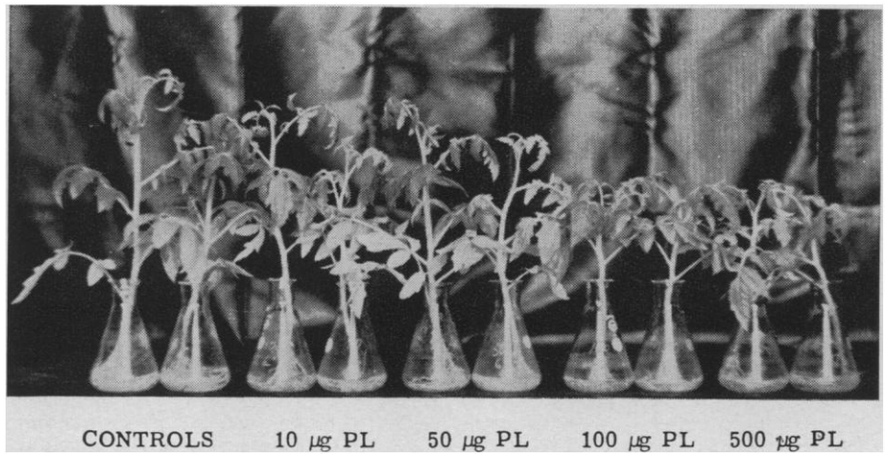

Fig. 1. Toxic effect of increasing amounts of polylysine on tomato cuttings. Duplicate cuttings were photographed 2½ weeks after being treated with 0, 10, 50, 100, or 500 μg of polylysine per milliliter of Hoagland's solution.

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Natural Lead-210 Content of Man

Abstract. The natural Pb^{210} content of ash samples from 18 cadavers has been measured, and an average in vivo content of 0.015 picocurie per gram of wet bone is estimated. The contribution of Pb^{210} to the radioactive dose from natural sources is about one-fifth that from internally deposited Ra^{226} and its daughters.

The possible overexposure of man to radiation from artificial sources has heightened interest in his inevitable exposure to radiation from natural sources and in the question of whether this natural radiation produces detectable biological effects. The external radioactive environment (*1*) can be measured with comparatively little difficulty. Although the identification and measurement of the radioactive trace constituents within the body of man present a greater problem, notable advances have been made in the last 10 years.

The body content of Ra^{226} has been measured by various investigators (*2*) in the United States and abroad. Measurements of K^{40} as a function of age have recently been reported (*3*). The study reported here (*4*) supplements the available data by supplying measurements of Pb^{210} content of 18 cadavers obtained locally.

At least two 10-g aliquots from the whole body ash of each cadaver were analyzed for Po^{210}. This measurement, converted to disintegrations per unit time, is equivalent to the Pb^{210} activity, since the attainment of secular radioactive equilibrium for the Pb^{210} to Bi^{210} to Po^{210} decay sequence depends on the 139-day half-life of Po^{210}, and since the material was ashed 8 years prior to the polonium measurement. The method of polonium analysis was that described by Black (*5*) and involves a nitric-perchloric acid digestion of the ash, plating out of the polonium on a silver foil, and counting in a low-background, gas-flow, proportional counter.

The results are listed in Table 1. The figures in column 3 have been corrected by subtracting reagent blanks (= 0.061 count/min). Since the overall recovery and counting efficiency, as determined by spiking with standard polonium solutions, was found to be 46 percent, the counts per minute are equal to picocuries of activity. Since Po^{210} is in equilibrium with Pb^{210}, the data in column 3 may be interpreted as picocuries of Pb^{210} per 10 g of whole body ash.

In calculating the in vivo body content of Pb^{210} (Table 1, col. 4), it was assumed (i) that all of the lead resides in the skeleton during life and that the wet skeletal mass is three times the whole body ash, and (ii) that all of the radon formed from Ra^{226} during storage escaped from the container. The Pb^{210} at sampling time is therefore corrected for decay to the time of death. The values listed are therefore maximal estimates of the in vivo lead content if we assume that none of the lead is lost in the cremation process, as seems likely to be the case (*6*).

An alternative calculation of the in vivo Pb^{210} content can be made. In this case the lead measured is assumed to be derived from three sources; it includes (i) that taken in with natural lead from the environment (in food, water, or air); (ii) the portion formed in vivo as a daughter of radon (30 percent equilibrium with body Ra^{226}), it being assumed that no excretion of lead occurs; and (iii) the lead formed during storage in the container by the Ra^{226} content of the ash, it being assumed that no radon escapes.

The contribution to the measured value from sources (ii) and (iii) can be readily calculated, inasmuch as the Ra^{226} content of the ash and its history are known. The remainder of the measured value may be corrected for decay to the date of death and assigned to source (i). These manipulations permit estimation of the total in vivo body content of Pb^{210}. The average of these estimates is two-thirds the average of the data entered in column 4, Table 1.

In extending these measurements to an estimation of the natural radioactive dose component from Pb^{210} and its daughters, the maximum estimate of average lead content is used as a basis. It is assumed that the lead decays in bone and that no translocation or excretion of the bismuth daughter occurs. It turns out that these assumptions are of relatively little importance except as they affect the site of production of the alpha-particle-emitting Po^{210} daughter of bismuth, since this isotope is the principal contributor to the dose. Black (*5*), on the basis of polonium measurements on mice exposed to radon 90 to 100 days prior to death, estimates that approximately 50 percent of the polonium produced from Pb^{210} remains in the bone. On the basis of this estimate, the in vivo polonium content is calculated to be 0.0075 pc per gram of wet bone, a value which is in reasonable agreement with Black's measurements (*5*) on single bone samples of unexposed human beings and animals, which range from 0.0036 to 0.034 pc.

The dose calculation, based on the above assumptions and on a relative biological effectiveness factor of 10 for alpha particles, yields an estimated yearly dose of 7.4 mrem, less than 1.5

Table 1. Natural body burden of Pb^{210}.

Age at death (yr)	Total ash wt. (g)	Po^{210} net (count/min 10 g)	Total Pb^{210} in vivo (pc)	Pb^{210} in vivo pc/g of wet bone
48	4410	0.48	302	0.023
85	1500	.32	66	.015
60	2500	.24	82	.011
57	2100	.14	40	.006
77	1700	.42	102	.020
76	2000	.25	72	.012
83	2200	.47	143	.022
77	790	.35	38	.016
36	2480	.14	47	.0063
74	2260	.47	151	.022
81	1570	.15	35	.0073
66	1970	.27	75	.013
85	2570	.46	165	.021
75	2000	.17	48	.008
74	2310	.11	37	.0053
33	3190	.44	188	.020
57	2500	.71	238	.032
32	1105	.38	56	.017
Average				0.015