of a histochemical reaction. For example we have bridged osmium through TCH to copper or mercury which is localized by chelation to the azo dye formed at the sites of aminopeptidase activity (*8*). The labeling of sites of aminopeptidase activity in rat kidney with osmium black is shown in a light micrograph, Fig. 6, and an electron micrograph, Fig. 7.

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References and Notes

1. J. S. Hanker, A. R. Seaman, L. P. Weiss, H. Ueno, R. A. Bergman, A. M. Seligman, *Science* **146**, 1039 (1964); A. M. Seligman, *Proc. Int. Congr. Histochem. Cytochem. 2nd* (Springer-Verlag, Berlin, 1964), p. 9.
2. A. M. Seligman, J. S. Hanker, H. Wasserkrug, H. Dmochowski, L. Katzoff, *J. Histochem. Cytochem.* **13**, 625 (1965).
3. A. M. Seligman, H. L. Wasserkrug, J. S. Hanker, *J. Cell Biol.*, in press.
4. L. A. Sternberger, E. J. Donati, C. E. Wilson, *J. Histochem. Cytochem.* **11**, 48 (1963).
5. L. A. Sternberger, J. S. Hanker, E. J. Donati, J. P. Petrali, A. M. Seligman, *J. Cell Biol.*, in press.
6. J. Chatt and F. A. Hart, *J. Chem. Soc.* **1960**, 2807 (1960).
7. D. P. Mellor and L. Maley, *Nature* **159**, 370 (1948).
8. M. M. Nachlas, B. Monis, D. H. Rosenblatt, A. M. Seligman, *J. Biophys. Biochem. Cytol.* **7**, 261 (1960); B. Monis, H. Wasserkrug, A. M. Seligman, *J. Histochem. Cytochem.* **13**, 503 (1965).
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Mycotoxicoses: Toxic Fungi in Tobaccos

Abstract. *Cigarette, cigar, and pipe tobaccos contain* Alternaria *spores and dematiaceous mycelia, with cigarette tobaccos being more heavily laden. A smoke aerosol generated from hay on which the fungus had been cultured caused, among other pathologic changes, pulmonary emphysema in mice.*

Various constituents of cigarette smoke have been studied as possible causes of pulmonary disorders (*1*), but mycotoxins have not been investigated. Random samples of 252 tobaccos from various brands of cigarettes and 50 each from cigars and pipe mixtures were examined mycologically; two predominating fungal isolates, a species of *Alternaria* and an *Aspergillus niger*, were subjected to preliminary toxicity tests in ICR mice by the oral and smoke aerosol routes.

Stereomicroscopic examination revealed no active fungal proliferation. Microscopic examination indicated past fungal growth, particularly in cigarette tobaccos (Fig. 1) which were considerably more heavily laden with *Alternaria* spores and corresponding dematiaceous mycelia than tobaccos from cigars or pipe mixtures (Table 1). The recovery, by culture, of viable fungi was very low, particularly in cigarette tobaccos.

The *Alternaria* and *Aspergillus niger* were grown at room temperature on Czapek's solution agar in petri plates, and aqueous homogenates were prepared by macerating, in a Ten Broeck tissue grinder, one part of fungal substratum with two parts of distilled water. Each of five mice received daily by stomach tube 1.0 ml of a respective homogenate until death occurred or for a maximum of 3.5 weeks. *Alternaria* homogenate produced progressive hypotonia after 24 hours, subepidermal hemorrhages by the 7th day, anorexia and pronounced cachexia by the 9th day, and death in all mice by 3.5 weeks. *Aspergillus niger* was not as toxic as *Alternaria*. Five control mice that received noninoculated agar homogenate daily for 3.5 weeks remained normal.

Timothy hay adjusted to approximately 20 percent moisture in Fernbach flasks, autoclaved (1 atm for 45 min), was inoculated with fungus and incubated for 4 weeks at 24°C; the culture was air-dried, pulverized, adjusted to approximately 20 percent moisture with 4 percent aqueous glycerol, and tested for smoke aerosol toxicity. Noninoculated, sterilized timothy hay, similarly treated, served as a control. The smoking apparatus consisted of a smoking pipe bowl attached by a piece of gum tubing to a glass tube which extended through a two-hole stopper into the bottom of a glass test tube, 65 by 500 mm. A piece of gum tubing attached to a glass tube extending through the stopper 5 cm into the test tube was connected to a vacuum source. Mice were placed in the smoke chamber, and, after a slight negative pressure was attained, the fungus substratum in the pipe was ignited and smoke was allowed to fill the chamber. Exposure time was 7 seconds daily until death occurred, or for a maximum of 466 days.

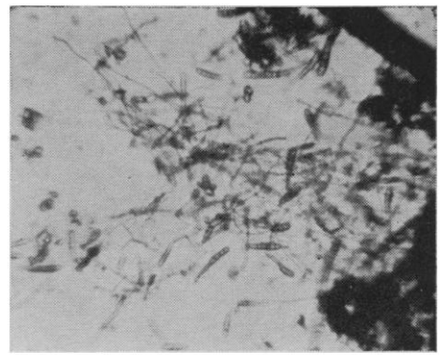

Fig. 1. Proliferative dematiaceous mycelia and dictyospores of *Alternaria* species in heavily contaminated cigarette tobacco. KOH preparation (about × 65).

After the 5th month, four of four mice challenged with *Alternaria* smoke developed progressive hypotonia and cachexia; in addition, from the 9th month until death ensued, there was lachrymation, edema, serum exudation with subsequent encrustations, and loss of hair about the face. Gross examination of the first mouse, killed on day 120, showed pulmonary congestion, edema, and emphysema. The second, third, and fourth mice died on days 315, 317, and 466, respectively; they

Table 1. Relative prevalence of fungal structures in tobaccos. Relative prevalence (R.P.) is designated as none (0); slight (1+); moderate (2+); heavy (3+); pronounced (4+).

Alternaria spores		Dematiaceous mycelia	
No.	R.P.	No.	R.P.
	252 Cigarettes		
32	1+	32	1+
100	2+	100	2+
92	3+	92	3+
28	4+	28	4+
	*Fungal score**		
2.46		2.46	
	50 Cigars		
34	0		
14	1+	38	1+
1	2+	7	1+
1	3+	4	3+
		1	4+
	*Fungal score**		
0.38		1.36	
	Pipe tobaccos		
6	0	3	0
26	1+	39	1+
9	2+	9	2+
3	3+	3	3+
6	4+	6	4+
	*Fungal score**		
1.54		1.60	

* Value for fungal score was obtained by multiplying, in each case, the number of samples containing the respective fungal structure by the relative predominance of the structure indicated in column "R.P." and dividing the sum of the products by the number of samples tested.

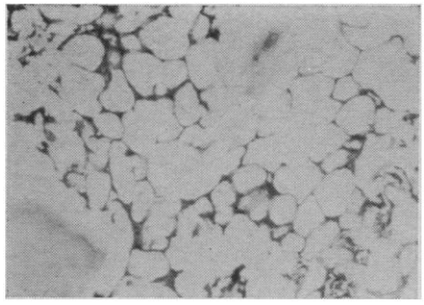

Fig. 2. Pulmonary emphysema in mouse after 317 days of exposure (7 sec/day) to *Alternaria* smoke aerosol. There is distention and attenuation of the alveolar walls (about × 25).

showed emaciation, thickening of the skin about the face, and congestion, edema, and emphysema of the lungs. Histologically, pulmonary emphysema was confirmed (Fig. 2) in the first three mice, two of which also evidenced alveolar cell carcinoma; all four showed secondary or terminal pneumonia, fatty infiltration or vacuolation (or both) and necrosis of the hepatic cells, and hemorrhage, congestion, tubular dilation, and glomerular interstitial nephritis in the kidneys. Four of six mice challenged with *A. niger* smoke developed pulmonary emphysema, among other pathologic changes, by the 8th month. Mice in the corresponding hay-smoke control groups remained normal clinically, and histologic examination of their tissues showed only pulmonary chronic inflammation.

Although *Alternaria* spores and mycelia are present in cigarette, cigar, and pipe tobaccos, there is a much higher percentage of heavy contamination in cigarette tobaccos. It appears significant that pulmonary emphysema was detected grossly in four of four mice exposed repeatedly to *Alternaria* hay-smoke aerosol, and in four of six mice exposed to *A. niger* smoke. Two mice in the *Alternaria* group developed alveolar cell carcinomas, but this incidence may have been fortuitous in that such neoplasms can occur spontaneously.

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Reference

1. *Smoking and Health*, Public Health Service Publ. No. 1103, (U.S. Dept. of Health, Education, and Welfare, 1964).

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Growth-Hormone Deficiency in Man: An Isolated, Recessively Inherited Defect

Abstract. *A deficiency of human growth hormone not associated with other pituitary deficiencies was observed in midgets with sexual ateliosis, a form of dwarfism inherited as an autosomal recessive trait. Body proportions, sexual development, birth weight, and postpartum lactation are normal in this syndrome.*

Gilford (*1*) termed normally proportioned dwarfs, or midgets, ateliotic, and he distinguished sexual and asexual types, depending on the state of sexual development and function. Autosomal recessive inheritance of sexual ateliotic dwarfism was supported by observations of affected sibs, both male and female, with unaffected parents who frequently were related (*2*).

At the same time as Gilford's nosologic contributions, the role of the pituitary in growth became apparent from clinical and experimental observations, and deficient production of a growth-promoting factor by the pituitary was suspected in ateliosis. In the absence of specific methods for assay of growth hormone, as well as the lack of potent, nonantigenic material with which a convincing therapeutic test could be made, pituitary insufficiency could be established only in asexual ateliotics, that is, cases of panhypopituitarism in which the additional deficiency of thyrotropic, adrenocorticotropic, and gonadotropic hormones indicated the pituitary basis of the defect in growth. Probably partly because the cases of panhypopituitarism were almost always nonfamilial (*3*), cases of sexual ateliosis were relegated to an idiopathic group called "primordial dwarfs," the tacit implication being that the defect is not pituitary but is, vaguely conceived, a genetically determined one "at the cellular level."

An isolated growth hormone deficiency has been suspected in some instances (*4*). The experience reported here documents an isolated deficiency of growth hormone in midgets with autosomal recessive sexual ateliosis.

Six sexually mature individuals with proportionate dwarfism were studied. Three (family A) were members of an inbred West Virginia kindred and three (family B) were husband, wife, and daughter (Fig. 1). Their ages ranged from 39 to 77 years, their heights from 123 to 139.5 cm, and their weights from 30.6 to 42 kg. None had a congenital malformation. Bone age was adult in all six. Birth weights, from 2727 to 4545 g, had been normal in all; the midget daughter of two midget parents had a birth weight of 2727 g, cesarean delivery having been performed about 2 weeks before term. Growth retardation was first noted in the first 2 years of life. Five of the six grew gradually, with halt in growth soon after puberty. One of the males had a 20.5-cm growth spurt during puberty, which did not occur until age 25. Secondary sexual characteristics were normal, although puberty was delayed by 2 to 10 years in each. The skin was smooth and wrinkled and appeared abnormally thick. The voices were high-pitched, with a "small" timbre. None had experienced hypoglycemic attacks and all were in general good health.

Extensive clinical, radiologic, and endocrinologic investigations were performed in each. Pituitary trophic hormone production was assessed by the following methods. (i) Thyrotropin (TSH) was assessed by the serum protein bound iodine and I^{131} uptake; (ii) gonadotropin (FSH) by biological activity of urinary extracts with the mouse uterine-weight technique (*5*); (iii) adrenocorticotropin (ACTH) by the presence in the urine of 17-hydroxycorticosteroids and 17-ketosteroids under basal conditions, and on the day of, and the day after the administration of metapirone, 300 mg per 100 pounds of body weight being given every 4 hours for six doses (*5*); (iv) growth hormone (HGH) by a standard human growth hormone immunoassay (*6*) after insulin-induced hypoglycemia and arginine infusions, both potent means of stimulating growth hormone secretion (*6*, *7*). In all six subjects, insulin, given intravenously in a dose of 0.1 unit per kilogram of body weight, produced marked symptoms of hypoglycemia approximately 30 minutes after the insulin administration. Arginine (20 g in 500 ml of water) was infused intravenously over a 30-minute period. The males were given 2.5 mg of stilbestrol twice a day for 2 days prior to the arginine infusion (*7*). Control values for growth hormone were obtained from 15 healthy postpubertal

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