References and Notes

1. A. S. Crafts, *Translocation in Plants* (Holt, New York, 1961), pp. 74 and 147; R. S. Russell and D. A. Barber, *Ann. Rev. Plant Physiol.* **11**, 127 (1960).
2. Complete details of the method outlined here are to be published.
3. R. Handley, R. D. Vidal, R. Overstreet, *Plant Physiol.* **35**, 907 (1960).
4. B. S. Meyer and D. B. Anderson, *Plant Physiology* (Van Nostrand, New York, 1952), p. 235.
5. R. C. Smith, *Am. J. Botany* **47**, 724 (1960).
6. Toluene, 3080 ml; 100 percent ethanol, 920 ml; DPO (2,5-diphenyloxazole), 16 g; and POPOP (p-phenylene-bis-(5-phenyloxazole), 60 mg. Two drops of 6*N* HCl were added to each 20-ml counting sample.
7. G. W. Butler, *Physiol. Plantarum* **6**, 617 (1953); L. Ordin and L. Jacobson, *Plant Physiol.* **30**, 21 (1955).
8. R. Brouwer, *Koninkl. Ned. Akad. Wetenschap. Proc. Ser. C* **57**, 68 (1954); G. Stenlid, *Physiol. Plantarum* **2**, 350 (1949).
9. R. Brouwer, *Koninkl. Ned. Akad. Wetenschap. Proc. Ser. C.* **56**, 106 (1954); L. Jacobson, R. J. Hannapel, D. P. Moore, *Plant Physiol.* **33**, 278 (1958).
10. L. K. Wiersum, *Rec. Trav. Botan.* **41**, 1 (1948).
11. H. E. Hayward and W. B. Spurr, *Botan. Gaz.* **105**, 152 (1943).
12. O. Biddulph, S. Biddulph, R. Cory, H. Koontz, *Plant Physiol.* **33**, 293 (1958).
13. R. Bledsoe, C. L. Comar, H. C. Harris, *Science* **109**, 329 (1949); M. H. Zimmermann, *Ann. Rev. Plant Physiol.* **11**, 167 (1960).
14. E. G. Bollard, *Ann. Rev. Plant Physiol.* **11**, 141 (1960).
15. O. Biddulph, F. S. Nakayama, R. Cory, *Plant Physiol.* **36**, 429 (1961).
16. P. J. Kramer, in *A Conference on Radioactive Isotopes in Agriculture* (U.S. Atomic Energy Commission, TID 7512 1956), p. 287.
17. P. R. White, *Am. J. Botany* **25**, 223 (1938).
18. L. C. Curtis, *Phytopathology* **34**, 196 (1944).
19. L. Jost and E. Reiss, *Z. Botan.* **31**, 65 (1937); P. Pilet, *Ber. Schweiz. Botan. Ges.* **61**, 410 (1951); *Les Phytohormones de Croissance* (Masson, Paris, 1961), p. 428.
20. G. E. Briggs and R. N. Robertson, *New Phytologist* **47**, 265 (1948); A. B. Hope and P. G. Stevens, *Australian J. Sci. Res. Ser. B* **5**, 335 (1952).

21. I am greatly indebted to Roy Overstreet, Louis Jacobson, and Cornelius A. Tobias for their guidance and constructive criticism during the course of this work. I also wish to thank Raymond Handley and Burton E. Vaughan for their suggestions.

26 December 1963

Toxin from Aspergillus flavus: Production on Food Materials of a Substance Causing Tremors in Mice

Abstract. *A strain of* Aspergillus flavus *produces, on foodstuffs, a substance which causes tremors and convulsions when administered to mice and certain other animals. The tremorgen, which differs from other recognized toxins attributed to this species, is associated with a distinct, dark spot on thin-layer chromatograms viewed under ultraviolet light.*

Strains of *Aspergillus flavus,* when cultured under various conditions, have been reported to produce one or more metabolites toxic for animals. One of these substances, aspergillic acid, was characterized by White and Hill (*1*). While studying methods of purifying the antibiotic flavicin, Bush and his co-workers (*2*) isolated two toxins from culture filtrates, fractions A and B. Fraction A was later identified as β-nitropropionic acid (*3*); fraction B was a yellow crystalline product differing from aspergillic acid in certain physical and toxicological properties. Kojic acid, first isolated from the traditional fermenting rice of the Orient, "koji," is a product of various strains of *Aspergillus,* including *A. flavus* (*4*). We reported that this neurotoxic antibiotic was produced abundantly on corn and other cereal grains inoculated with *A. flavus,* but only traces were detected in animal feeds naturally contaminated by the fungus (*5*). Oxalic acid was also produced by this species on certain feeds (*6*) and often appeared simultaneously with kojic acid in liquid cultures of the fungus (*5*).

English and Dutch workers (*7*) extracted and crystallized two potent hepatotoxins from peanut meal contaminated with *A. flavus.* These were designated "aflatoxins B and G" (proposed B_1 and G_1), according to their respective blue and green fluorescence on paper or thin-layer chromatograms. Asao *et al.* (*8*) presented evidence concerning the molecular structure of these toxins, showing their relationship to synthetic coumarin. A recent report (*9*) described another aflatoxin, designated B_2, which apparently is dihydroaflatoxin B_1.

Milner and Geddes (*10*) reported strains of *A. flavus* to be among the most common fungal contaminants of cereal grains. In our experience this organism has been a frequent isolate from toxic feed, particularly from samples of contaminated feed connected with liver disease syndromes of swine and cattle similar to those described by Burnside *et al.* (*11*).

In a program of screening toxigenic fungus cultures, we found that *A. flavus,* strain QM 6738, when grown on moistened cracked corn, produced an unusual neurological toxin in addition to kojic acid. Both substances were present in crude methanol and chloroform extracts of the experimentally contaminated feed. This new toxin differed from kojic acid and the other recognized toxins of *A. flavus* in certain chemical properties and physiological effects. The characteristic response in mice consisted of tremors, sometimes followed by convulsions, depending upon the dosage. Several other isolates of *A. flavus,* including two which produce aflatoxins, failed to have this effect.

Dublin ICR and SW mice showed signs of illness 10 to 30 minutes after administration by stomach tube of 0.5 to 1.0 mg of the partially purified toxin. This dose represented approximately a 50-fold increase in toxicity over unfractionated methanolic culture extracts. The animals first became inactive but responded to auditory and tactile stimuli and exhibited marked tremors of the entire body when movement was attempted. The trembling became more pronounced in 1 to 2 hours and continued for several hours. Marked improvement was usually evident the day after administration of the toxin, although some mice continued to exhibit tremors and stiffness of the limbs for 2 days. Somewhat smaller doses produced either no perceptible response or only temporary inactivity with mild tremors of short duration.

A dose of 2 mg resulted in characteristic tremors followed, in 1 to 2 hours, by sudden hyperactivity with intermittent convulsions. The slightest stimulus caused affected mice to make rapid paddling movements of the legs without forward progress. Frequently an animal would rise on its hind legs, fall backward, and make several twisting, "log-rolling" motions of its entire body before righting itself. Death sometimes occurred within 2 hours during a tetanic convulsion. Animals dying in convulsions showed marked rigidity of peripheral muscles, observable after death. Mice that survived the repeated convulsive seizures usually continued trembling for 1 to 2 days, but eventually recovered.

The response of mice to intraperitoneal injection was very similar to their response after administration by stomach tube. Guinea pigs and rats were also susceptible to either oral or intraperitoneal administration of the tremorgen.

In mice the response to this toxin is

somewhat similar to that elicited by tremorine (1,4-dipyrrolidino-2-butyne), but differs in several significant respects. The marked parasympathetic stimulation exhibited by tremorine is not evident; atropine and scopolamine given before or after administration of the toxin fail to prevent the tremors or convulsions. Methantheline, phenobarbital, and mephenesin also do not alter typical reactions. The unusual action of tremorine was emphasized by Everett *et al.* (*12*) who found that less than 10 of the 10,000 drugs they tested were capable of causing sustained tremors.

The tremorgenic toxin is formed during growth of *A. flavus* on oats, millet, rice, potatoes, or corn and is produced in considerable quantities when the fungus is grown on cracked, moistened corn at room temperature for 2 to 3 weeks. Very little or none of the toxin was detected on samples of timothy hay and peanut meal inoculated with this organism. The peanut meal is, however, quite suitable for the production of aflatoxin by strains of *A. flavus* which synthesize these hepatotoxins.

Strain QM 6738 of *A. flavus* characteristically forms numerous dark sclerotia which contain the toxin. Chloroform extracts of the conidia, however, do not contain detectable quantities. Production, extraction, and preliminary purification were accomplished by methods similar in some respects to those described for the hepatotoxin of *Penicillium rubrum* (*13*) and the aflatoxins of *A. flavus* (*14*), followed by chromatography on two successive columns containing layers of silica gel, florisil, and alumina. Florisil was used for selective removal of several blue and purple fluorescent materials in the crude extract. None of the substances was capable of causing tremors or convulsions in mice. Each step in the purification procedure eliminated significant quantities of nontremorgenic extract components.

On thin-layer silica gel plates developed with 3 percent methanol in chloroform, the tremorgen was associated with a nearly colorless spot which appeared as a dark area under ultraviolet light at about R_F 0.7 to 0.8. This differs from aflatoxins which fluoresce with a bluish-purple or green color when viewed on chromatograms. The toxin spot assumed a visible yellowish-brown color in 48 to 72 hours. If the material is sufficiently concentrated, a nonspecific brown color may be produced immediately after development by spraying with *m*-dinitrobenzene. A series of six or more successive thin layer chromatograms was required to eliminate all but the one spot attributed to the toxin. Some loss of toxin, attributable in part to incomplete separation of extract components on column and thin-layer chromatograms, occurred during purification.

The toxic residue, extracted with chloroform from thin-layer plate scrapings, was a yellowish-brown solid at room temperature. The addition of petroleum ether to a concentrated chloroform solution precipitated light yellow, amorphous, plate-like particles. These were readily soluble in several polar and nonpolar solvents but were only slightly soluble in petroleum ether and cyclohexane. Attempts are being made to obtain sufficient material for more extensive investigations.

BENJAMIN J. WILSON
Department of Biochemistry, School of Medicine, Vanderbilt University, Nashville, Tennessee

CHRISTINA H. WILSON
Microbiological Research Laboratory, David Lipscomb College, Nashville

References and Notes

1. E. C. White and J. H. Hill, *J. Bacteriol.* **45**, 433 (1943).
2. M. T. Bush, A. Goth, H. L. Dickison, *J. Pharmacol. Exptl. Therap.* **84**, 262 (1945).
3. M. T. Bush, O. Touster, J. Brockman, *J. Biol. Chem.* **188**, 685 (1951).
4. K. Saito, *Botan. Mag.* (*Tokyo*) **21**, 7 (1907).
5. B. J. Wilson and C. H. Wilson, *Bacteriol. Proc.*, 62nd Annual Meeting, Kansas City, Mo., 1963, p. 28.
6. ———, *Am. J. Vet. Res.* **22**, 961 (1961).
7. B. R. Nesbitt, J. O'Kelly, K. Sargeant, A. Sheridan, *Nature* **195**, 1062 (1962); A. S. M. Van der Zijden, W. A. A. Blanche Koelensmid, J. Boldingh, C. B. Barrett, W. O. Ord, J. Philp, *ibid.*, p. 1060.
8. T. Asao, G. Büchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, G. N. Wogan, *J. Am. Chem. Soc.* **85**, 1706 (1963).
9. S. B. Chang, M. M. Abdel-Kader, E. L. Wick, G. N. Wogan, *Science* **142**, 1191 (1963).
10. M. Milner and W. F. Geddes, in *Storage of Cereal Grains and Their Products*, J. A. Anderson and A. W. Alcock, Eds. (Am. Assoc. Cereal Chemists, St. Paul, Minn., 1954), p. 165.
11. J. E. Burnside, W. L. Sippel, J. Forgacs, W. T. Carll, M. B. Atwood, E. Dol, *Am. J. Vet. Res.* **18**, 817 (1957).
12. G. M. Everett, L. E. Blockus, I. M. Shepperd, *Science* **124**, 79 (1956).
13. B. J. Wilson and C. H. Wilson, *J. Bacteriol.* **84**, 283 (1962).
14. K. Sargeant, J. O'Kelly, R. B. A. Carnaghan, R. Allcroft, *Vet. Record* **73** (**1961**), 1219 (1961).
15. This study was supported in part by grant E-4682 from the National Institute of Allergy and Infectious Diseases and by a grant from the Geschickter Fund for Medical Research. We thank Dr. E. G. Simmons, Quartermaster Research and Engineering Command, U.S. Army, for supplying the strain of *A. flavus*, and Professors W. J. Darby and Oscar Touster for suggestions.

17 February 1964

Radioiodine Metabolism in Children and Adults after the Ingestion of Very Small Doses

Abstract. *One day after oral ingestion of very small doses (about 0.01 microcurie) of an I^{131}/I^{125} mixture, the radioiodine content of the thyroid gland was similar in children and adults (about 20 percent of the dose ingested) as was the biological half-life of the iodine (about 90 days). Measurements were made in 15 minutes by means of a whole-body counter with a cylindrical sodium iodide (Tl) crystal, 20 cm in diameter and 10 cm thick.*

Radioiodine released by nuclear explosions can reach the food chain, become deposited in the human thyroid gland, and increase the radiation dose this organ normally receives. To estimate this dose one requires knowledge of the retention of radioiodine by the thyroid gland as a function of time. Data on children are scarce, partly because of the reluctance of experimenters to use traditional tracer techniques on the growing and (presumably) more radiosensitive thyroid gland; for such techniques, doses of up to 25 μc of radioactive iodine must be ingested. However, we devised recently a new, highly sensitive technique which makes possible the use of very small doses of tracer (about 0.01 μc). By this method we have shown that the percentage of the dose of radioactive iodine administered which is present in the thyroid gland 1 day later is similar for children and adults. A preliminary description has been given (*1*); we describe it briefly in this report.

We use a large NaI (Tl) crystal of the type generally used for estimating radioactivity in humans (*2*). Our crystal, which measures 20 cm in diameter and is 10 cm thick, has a 0.0127-cm aluminum entry window which admits radiation of low energy. It is placed a short distance (8.2 cm) from the subject's neck to increase sensitivity. At this distance, however, uncertainty of the depth of the thyroid in the neck can produce a significant error. To determine and to correct for this, a double tracer technique was developed. A mixture of I^{131}, with a half-life of 8 days (emitting mainly 364-kev γ-rays), and I^{125}, with a half-life of 60 days (emitting mainly 27-kev x-rays) was administered orally, nominally 0.01 μc of each. Because of this