Toxin-Producing Aspergillus Isolated from Domestic Peanuts

Abstract. *Nine species of fungi isolated from stored domestic peanuts were grown in the laboratory on sterilized peanuts and were incorporated into diets fed to ducklings. Symptoms of acute toxicity resulted only after consumption of one of the diets and this one contained material incubated with the fungus,* Aspergillus flavus *Link ex Fries.*

An investigation of fungi for toxin production was initiated after reports (*1–3*) that feeding certain peanut meals to turkey poults, ducklings, and other animals produced harmful effects attributable to a toxin produced by the fungus *Aspergillus flavus* Link ex Fries. That liver tumors are induced in rats by feeding certain peanut meals has been reported previously in England (*1*) and the United States (*4*).

Nine species of fungi, isolated from domestic peanuts in 1957 (*5*) and maintained on Czapek's solution agar containing 20 percent sucrose, were cultured on autoclaved shelled peanuts, *Arachis hypogaea* L. variety Early Runner (*6*). These species were *Aspergillus flavus, A. candidus* Link ex Fries, *A. ruber* (*Eurotium rubrum* Brewer), *A. amstelodami* (*E. amstelodami* Mangin), *A. chevalieri* (*E. chevalieri* Mangin), *A. restrictus* G. Smith, *A. micro-virido-citreus* Costantin and Lucet, *A. terreus* Thom, and *Penicillium citrinum* Thom. After a 3-week incubation period, the nine lots of fungus-inoculated peanuts and an uninoculated control were oven dried at 135°C for 4 hours and extracted with petroleum ether. These treatments killed the fungi in the inoculated material.

The ten lots of defatted residues were ground and mixed into individual test diets. The percentages of ingredients in the test diets were defatted peanut residues, 40; casein, 8; gelatin, 5; alphacel, 3; sucrose, 13.6; beef tallow, 18; salts, 6; vitamin premix, 4; choline chloride, 0.4; and cod liver oil, 2. White Peking ducklings, 2 days of age, were placed in electric brooders and fed on a commercial "starter" for 2 days. They were then separated into ten groups of five ducklings each; and the ducks had free access to the test diets. Weights were recorded after 3 days (see Table 1).

All groups, except the one fed the diet containing *A. flavus*, gained weight and were normal in appearance. The groups fed *A. terreus, A. restrictus*, and *P. citrinum* gained more, and the one fed *A. ruber* gained slightly less than the control group. The ducks in the group fed *A. flavus* lost weight and they had a very unhealthy appearance. After the 3rd day the control diet and some of the other diets were exhausted. All groups except the one fed *A. flavus* were discontinued. This group was continued on the same diet; the mortality was five out of five and the average length of survival was 5 (range 4 to 7) days.

In order to obtain a further test of the toxicity of the diet containing the *A. flavus*-inoculated material, the control group was changed to this diet after the 3rd day. All five of the ducklings in this group died and the average survival time was 3 (range 1 to 5) days after they were placed on the toxic diet; the average final weight was 44 g less than it was prior to being placed on the diet containing the peanut residue inoculated with *A. flavus*. These birds had been without food for several hours before they were changed to the toxic diet which they consumed greedily. This may explain their shorter survival time as compared with the original group fed this same diet. Ducklings from the same hatching fed a similar diet containing a nontoxic peanut meal survived in good condition for a period of 22 weeks.

The changes observed at autopsy were a marked yellow discoloration of the liver, accompanied in a few instances by flame-shaped areas of hemorrhage. Microscopic examination of tissue preparations revealed marked histopathologic changes in the liver and kidney. In the liver focal areas of necrosis, which often destroyed relatively large areas of the hepatic parenchyma, were consistently observed. The liver as a whole showed cellular disorganization. In the hepatic tissue adjacent to the periportal areas (Fig. 1), the cells showed cytoplasmic and nuclear vacuolization; they were basophilic when stained with hematoxylin and eosin, and they were often arranged in ductules whose structures resembled bile-ducts. The liver cells comprising the remainder of the lobule were acidophilic and showed a homogeneous cytoplasm. There was a generalized activation of reticuloendothelial elements.

In the kidney hyperplasia and sclerosis of the glomeruli were widespread. These were accompanied by a marked reticuloendothelial hyperplasia that was primarily restricted to the cortex. Focal areas of tubular distention were observed and a few arterial vessels showed a severe arteritis with exudate filling the lumens. All spleens were markedly congested and germinal centers were not apparent. There was increased activity among the interacinar cells of the pancreas, and many islets of Langerhans appeared hyperplastic.

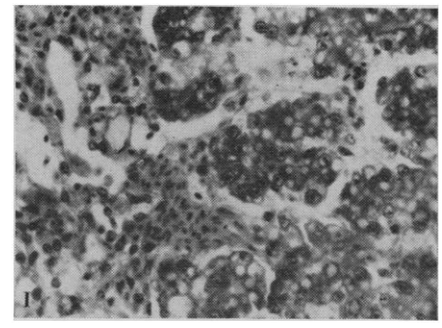

Fig. 1. Photomicrograph illustrating the parenchymal and perivascular reaction in the livers of ducklings fed peanut meal contaminated with toxins of *A. flavus*. These changes occur primarily in the liver parenchyma adjacent to the portal tract and are characterized by ductule-formation, cytoplasmic vacuolization, increased mitotic activity, and focal liver-cell necrosis as well as enlargement and activation of the littoral cells lining the sinusoids. Bouin fixation; hematoxylin-eosin stain; approximately × 1200.

The toxic meal was examined by

Table 1. Weight change of ducklings fed defatted residues of peanuts incubated with pure cultures of various species of *Aspergillus* and *Penicillium* for 3 days.

Species of fungus	Average initial weight per bird (g)	Average weight gain per bird (g)
A. amstelodami	79	64
*A. flavus**	79	—13
A. chevalieri	79	58
A. ruber	79	53
A. terreus	79	70
A. restrictus	80	75
P. citrinum	81	71
A. candidus	80	61
None (control)†	80	60
A. micro-virido-citreus	80	63

* All the ducklings died; the average survival time was 5 (range 4 to 7) days. † The five ducklings were transferred from the control diet to the diet containing *A. flavus* after 3 days.

procedures described (*2*) and contained a blue fluorescent material having the properties of aflatoxin. Thus, it appears that an aflatoxin-producing strain of *A. flavus* has been isolated from domestic peanuts (*7*).

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References and Notes

1. M. C. Lancaster, F. P. Jenkins, J. Philp, *Nature* **192**, 1095 (1961).
2. K. Sargeant, A. Sheridan, J. O'Kelly, R. B. A. Carnaghan, *ibid.*, p. 1096.
3. F. D. Asplin and R. B. A. Carnaghan, *Vet. Rec.* **73**, 1215 (1961).
4. W. D. Salmon and P. M. Newberne, *Cancer Res.* **23**, 571 (1963).
5. U. L. Diener, *Phytopathology* **50**, 220 (1960).
6. H. S. Ward, Jr., and U. L. Diener, *ibid.* **51**, 241 (1961).
7. Supported in part by U.S. Public Health Service grant CA 01018 from the National Cancer Institute.

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Leukemia, Multiple Myeloma, and Aplastic Anemia in American Radiologists

Abstract. *A survey of 425 death certificates of radiologists dying between the ages of 35 and 74 during the years 1948 to 1961 reveals a statistically highly significant excess of deaths from leukemia, multiple myeloma, and aplastic anemia. That this excess is due to radiation exposure (or to some factor acting in a similar manner), rather than to an artifact of diagnosis is suggested by the absence of deaths ascribed to chronic lymphatic leukemia.*

It has never been excluded that the excessive number of deaths from leukemia in American radiologists (*1–3*) is largely or wholly an artifact of diagnosis. For example, leukemia might have the same probability of occurrence in a radiologist as in a member of the general male population, but have a higher probability of being accurately diagnosed in a radiologist owing to such factors as his more ready access to medical facilities. A way of testing such a possibility became evident when Court Brown and Doll (*4*) discovered that one form of the disease, namely, chronic lymphatic leukemia (CLL), is apparently either not induced by ionizing radiation, or is much less readily induced than are the forms of this disease in which other types of cells are affected. Their finding was based on leukemia deaths arising in a group of adult British males who had received x-ray therapy for an arthritic condition (ankylosing spondylitis). Among 28 such deaths only one was reported as due to CLL; yet, as will be discussed below, this type of leukemia is one of the commonest forms of the disease in adult white males. More recently, Pochin (*5*) cites only one death reported as being due to lymphatic leukemia (subchronic) among 17 deaths from leukemia arising in a group of adults who had received radioiodine therapy for hyperthyroidism.

Evidently, if radiation (or some other agent acting in a similar manner) is responsible for a rise in the death rates for leukemia in a given population, the death rate for chronic lymphatic leukemia in that population should rise little, if at all. On the other hand, if diagnosis is responsible, the death rate for CLL should rise proportionately, since diagnosis ought not to affect classification by histological cell type differentially.

This report presents the principal findings of a study designed to answer three interrelated questions. (i) Do excessive numbers of deaths from leukemia continue to occur in American radiologists in recent years? (ii) If so, does the number of deaths from CLL occur in accord with expectation based on radiation or on diagnosis as the responsible factor? (iii) Do excessive numbers of deaths occur in this group from diseases related to leukemia?

To assess the significance of an observed number of deaths from a given cause it is first necessary to determine the composition, with respect to age and size, of the living population that produces such deaths, and then to compute the number of deaths expected in that population had it been subject to the mortality of some standard reference population. The living population of radiologists chosen for study is restricted to those physicians who are listed in the biennial editions of the *Directory of Medical Specialists* (DMS) (*6*) as being certified by the American Board of Radiology. Punched cards showing name, year of Board-certification, and year of birth were prepared for all such individuals residing in the continental United States and having entries in the 1950 and 1960 editions of these directories (*7*). Similar cards, showing also year of death, were prepared for Board-certified radiologists who were known to have died in the study period and whose names appeared in one or more of the DMS editions spanning the years 1948 to 1960, inclusive. The resulting deck of cards, after elimination of duplicates and of cards bearing female names, provided the basic data for computing the composition, with respect to age, of the living male population as of July 1 of each year of the 14-year period, and for each year of age from 35 to 74, inclusive (*8*). The results, by 5-year age groups, for representative years, 1950, 1955, and 1960, are shown in Table 1. The estimated number of male radiologists aged 35 to 74 years, inclusive, increased from 2167 in 1948 to 4713.5 in 1961; for the entire 14-year period the number of man years at risk at these ages was 47,348.

The U.S. white male population was the standard chosen for the present study. It is the ultimate population from which the radiologists are drawn and it is the only relevant population for which sufficient data are available to calculate death rates for the rather rare diseases here under study.

Death rates for leukemia and related diseases categories in the U.S. white

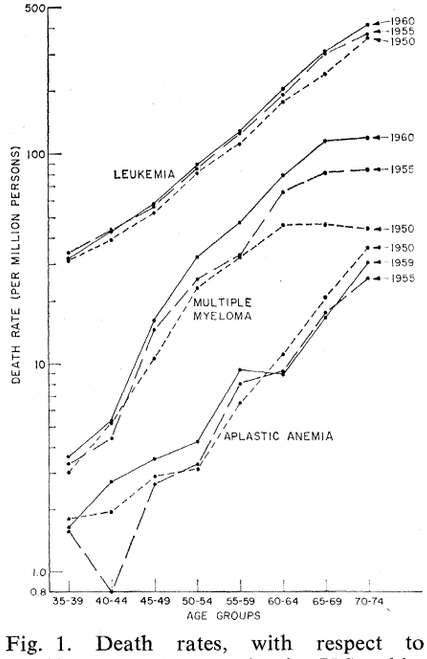

Fig. 1. Death rates, with respect to specific age and cause, in the U.S. white male population (deaths per million living persons per year) for selected years of the study period (semi-log plot) (*11*). Death rates for aplastic anemia for each age group below age 50, and for multiple myeloma for each group below age 40, are based upon 20, or fewer, deaths.