Hazes of Mycotoxins to Public Health

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ABSTRACT

Since the discovery of aflatoxins, the ultimate question concerning mycotoxins in general is whether they relate to human as well as animal health. Sources of information on this question include results of several food and health surveys done in various countries. These studies have examined the possible relationship of mycotoxin contamination of foodstuffs and the incidence of endemic human diseases in geographically defined regions. In addition, reports linking toxin contamination of dietary items and isolated cases of disease have also come from certain countries. Most of these reports have been concerned with aflatoxins which are among the more easily detected mycotoxins in foods. Suitable analytical methods for the more obscure mycotoxins are sorely needed. Certain recognized diseases which might be attributable to mycotoxins require further investigation. Factors favoring mycotoxin contamination of human foods are usually quite obvious, involving improper harvesting and methods of food storage that favor fungus contamination and growth. Use of fermented foods and beverages by native populations may offer certain risks to health; the role of regional food customs and climatic factors favoring fungus contamination of foods are also important. Proper inspection and control of food by governmental agencies as protective measures should be emphasized.

Before discovery of the aflatoxins, the question was frequently asked whether toxins played a significant role in diseases of livestock and other animals. The pioneering work of Forgacs and his associates (20) in the United States and Mortimer, Taylor, Hodges, and others (54) in New Zealand helped to answer this question at least for certain animal diseases. After discovery of aflatoxins and demonstration of their carcinogenicity in laboratory species, the next important question frequently asked was, "Are mycotoxins involved in human health?" The answer, we know now, is a definite, "Yes". However, the proper response to a logical follow-up question is not so clear; namely, "To what extent are mycotoxins, particularly aflatoxins, affecting human health?" The importance of these queries is obvious when one considers that environmental agents may be causing many of the neoplasms of man (48). Of course, our concern with mycotoxins is not confined to their carcinogenic potential since many different tissues and organs of susceptible animals may be targets of the wide variety of fungus toxins now recognized, and several disease manifestations may ensue from their toxic action.

It is obvious that an assessment of human susceptibility to mycotoxins is difficult to make since we dare not purposely employ humans in toxicological experiments. Thus, investigators have attempted to extrapolate animal data to man, realizing that species variations often make such data of limited value. The use of sub-human primates is generally thought to allow for more meaningful extrapolations. Perhaps most useful estimates of man's susceptibility and involvement are gained from analyzing the mycotoxin levels of human foods and observing the type and severity of diseases associated with their consumption (12).

Although ingestion of moldy cereals, vegetables, or nuts is probably the most important mode of contact with mycotoxins for both man and animals, other means of acquiring intoxications must also be considered. These include skin contact with certain dermally toxic compounds, consumption of contaminated meat or milk, and, on occasion, aerosol inhalation of dust containing fungus elements and toxins. Laboratory personnel working with toxigenic fungus cultures or their extracted toxins should be aware of the health hazards inherent in many experimental manipulations done in the laboratory.

As with animals, the nutritional status of man might be expected to play a role in his susceptibility to mycotoxins (42). The effect of this factor, however, is not always predictable on the basis of present knowledge derived from animal studies.

In highly civilized societies, food that is obviously moldy or that is organoleptically objectionable, is likely to be rejected. However, toxin may be present in certain foods or beverages that do not present these warning signs to the senses of sight, smell, or taste. As with animals, the element of hunger can be expected to favor ingestion of contaminated food by man where no alternative is available. In primitive societies food shortages, along with lack of knowledge as to how foods are contaminated, pose serious health hazards.
should be cultivated, harvested, selected for use, properly stored, or held after cooking can augment the intake of undesirable foods. Moreover, use of primitive fermentation procedures for both food and beverage could conceivably favor formation of microbial toxins. Conditions that lead to mycotoxin contamination of foods have been reviewed by Hesseltime (25).

Surveys designed to reveal the possible relationship of mycotoxins in food and human diseases are sometimes made less meaningful by the presence of other deleterious environmental agents. Specifically, one must consider practices such as use of toxigenic native medicinal plants which can produce pathological lesions somewhat similar to those caused by certain mycotoxins (16). Similarly, it is often difficult to differentiate among lesions caused by infectious agents, such as endemic infectious hepatitis virus, and hepatotoxic fungus metabolites.

**HISTORICAL ASPECTS**

By definition, classical ergot poisoning in man was also a mycotoxicosis. This being the case human involvement in fungus intoxications has a history dating back several centuries. Ergotism from food, which apparently can manifest itself by both a hallucinogenic state and by gangrene development in the limbs, has an interesting history. Somewhat belated recognition of cereal grain infected with *Claviceps purpurea* as the cause of this disease led to measures that have largely eliminated ergotism in man in most parts of the world.

The pictorially documented mass outbreak of hallucinogenic disease in Pont St. Esprit, France, in 1951 by *Life* magazine (4) was considered by some experts to be due to contamination of flour by a mercury compound rather than ergot as first commonly assumed. The unusual mass affliction and the prolonged trauma among those residents who consumed the bread of madness were the subjects of a book entitled, *The Day of St. Anthony’s Fire* (21). An extensive review of the problem of ergot and ergotism was provided by Barger (7). A condensed historical account, along with extensive coverage of the chemical compounds extracted from cultures of *C. purpurea*, have been written by Van Rensburg and Altenkirk (58).

The early interest of Japanese investigators in fungus toxins was based, in part, on the knowledge that mold-contaminated food had apparently caused illness and death, several decades ago, among groups of people in Japan and other rice-eating countries. For example, it is strongly suspected by several Japanese scientists that acute cardiac beriberi was related to use of moldy rice, and that a subsequent decrease in cases coincided with institution of rice inspection by the governments involved (56). Cardiac beriberi was frequently encountered in Japan in the latter part of the 17th century and beginning of the 18th century, but is now rarely seen in that country.

Uruguichi and coworkers (57) have documented outbreaks of disease in the Orient which may have been caused by moldy food: (a) In 1971 a large number of Korean immigrants in Tunghwang, Manchuria ate microbially damaged corn over a period of a few months, and many died as a result. Findings at necropsy included degenerative changes in the liver and heart. (b) In the autumn of 1952, within the suburbs of Tokyo, some 25 persons were made ill from eating rice contaminated with *Fusarium* sp. The illness which included nausea, vomiting, and drowsiness, was similar to outbreaks of poisoning occurring in other districts. This author speculated further that the high incidence of primary hepatic carcinoma in tropical countries of Asia was probably related to eating damaged rice.

Saito and Ohtsubo (43) described an outbreak of human disease occurring in Tochigi and Kochi prefectures of Tokyo in 1955 attributed to deteriorated rice containing toxigenic *Fusarium* species. More than 40 persons were affected, suffering nausea, vomiting, and diarrhea. A similar poisoning occurred in Hokkaido in the post-war years and in 1946 in Tokyo. Imported wheat flour containing *Fusarium graminearum* was isolated but not proven to be the toxigenic agent.

Most persons familiar with mycotoxins are probably well informed on the human mycotoxicoses that occurred in the Soviet Union during the second World War. Overwintered grain infected with *Fusarium sp.* was the cause of a syndrome known as alimentary toxic aleukia (ATA) (22). ATA developed in increasingly severe stages with the continued intake of toxic food. More recent studies have shown that the fungus isolates responsible for ATA are capable of producing trichothecenes (53), a fact that correlates well with the pathological picture in animals injected or fed the purified compounds. Stachybotryotoxicosis, a disease of horses and man that occurs in Russia and other countries, is caused by *Stachybotrys atra*, which also produces trichothecenes.

**AFITOXINS AS AGENTS OF HUMAN DISEASE**

Undoubtedly the initial discovery of aflatoxins in the 1960’s (13) and preliminary assessments of their toxic potential, especially knowledge of their carcinogenicity in rats (46), were major events leading to worldwide recognition of the importance of fungus toxins to both animal and human health. Soon thereafter the words “mycotoxin” and “mycotoxicosis” were coined, and their meanings gained considerable significance. A factor aiding all phases of research in aflatoxins is their remarkable fluorescence under long wave ultraviolet light. This property has enabled their detection as naturally occurring contaminants of several foods used by man or animals in many parts of the world (62). This property has also led to methods for quantitation of aflatoxin in food samples. The analytical data in turn have been related to the incidence or extent of liver disease in various human populations.

Public health implications for these compounds were realized early. Dr. Max Milner of UNICEF, of the United
Nations Organization, convened a meeting of interested scientists in New York City in October, 1962, to help assess the hazards of aflatoxin to human health. Under consideration was suspension of the ARLAC infant food program in Nigeria since the peanut meal component was likely at that time to be contaminated with these metabolites of *Aspergillus flavus*.

Since their discovery, aflatoxins, more than any other mycotoxins, have been considered as prime threats to health. This is especially true in tropical countries of the world where the humidity is high and living conditions are often primitive. In several of these areas post-necrotic cirrhosis of the liver and primary hepatoma show a relatively high incidence and appear to have an environmental etiology (33).

Data suggesting that aflatoxin\(^1\) was undoubtly ingested by population groups of several countries and was related to hepatic disease have come from three types of studies. The first of these consisted of surveys of various food commodities for toxigenic fungi and for aflatoxin levels. The frequency and levels of toxin in foods were then related to previously recorded incidence of human disease, mostly primary hepatoma, in the selected region. More specific information has been provided where individual prepared food samples were analyzed for toxin and compared to the hepatoma case rate. Where an individual's intake of aflatoxin could be determined, secretion of toxin in breast milk, or excretion in urine, were also sometimes measured. A second general source of information has come from unusual outbreaks of disease among groups of people in which the cause was apparently related to consumption of a common type of food that was mold-contaminated and contained measurable levels of aflatoxin. Thirdly, there have been reports of somewhat isolated cases where one or a few persons, usually children, have become ill after consuming aflatoxin-laden food. From these cases the aflatoxin levels of the food were determined which, along with knowledge of the period of ingestion, gave an estimate of total dosage.

Possible sources of erroneous information in each of these situations are obvious, but in spite of imprecise data collection methods, information has been gained indicating correlation between aflatoxin levels of foods and incidence of human liver diseases.

The following are brief resumes of some of the surveys, endemics, and individual cases that have been reported over the last decade from different geographical regions of the world.

**Uganda**

Alpert and coworkers (2) surveyed the incidence of primary hepatoma in various tribal regions of Uganda in 1966-1967. The incidence of liver disease, particularly liver cancer, was considered to be high in that country (1). Aflatoxin levels were determined in 480 food samples stored for consumption between harvests. Approximately 30% of these contained detectable levels of aflatoxins and 3.7% had more than 1 mcg/kg. The primary hepatoma rate was fairly uniform over most of the country, ranging from 1.4 to 3.0 cases per 100,000 population per year except for Karamoja region of the Northern Province which had a rate of 15.0. Of 105 food samples analyzed in this area, 43.8% were aflatoxin-positive which was significantly higher than for most other regions sampled. Assuming a daily food consumption of 500 g of staple grain, per capita ingestion in Karamoja could be on the order of 0.02 to 2.0 mg daily, a level known to be hepatotoxic to monkeys. Karamoja has a dry semi-desert climate whose annual rainfall is concentrated into a short rainy season once or twice a year, turning the semi-desert region into mud fields for several days or weeks. Thus, the microenvironment of the food storage area was considered to be a major factor determining mold growth.

The Toro region, containing the Bwamba tribe, had considerably higher frequency of aflatoxin as well as higher average amounts of aflatoxin in food samples. However, no data on cancer incidence were available for this region.

The authors believed that both poverty and food scarcity among tribes living in Karamoja also contributed to chronic ingestion of foods that were moldy and contained relatively high levels of aflatoxin. There was no appreciable difference in the aflatoxin content of food stored in the raw form or as milled or cooked preparations. Neither the type of storage nor its duration seemed to affect the aflatoxin content. Three of five foods fermented and stored for use in beer production were heavily contaminated.

**Taiwan**

The incidence of primary liver cancer has been high in Taiwan compared to that of the United States and Europe. The climate of the island is generally warm and humid which tends to favor mold growth on foods. Nearly all agricultural products are sun-dried in the field and stored in damp storehouses. A survey in 1966 of market rice, sweet potatoes, and peanuts revealed occasional contamination of sweet potatoes and peanuts by aflatoxin, but none was found in market rice samples. However, moldy rice samples found to contain aflatoxin-producing fungi were believed to have caused illness of 25 persons, among 39 members of three farm families from Shung-chi township of Taipei county. Three of the 39 died of undiagnosed illness. This report by Tung and Ling (55) offers only very speculative information on the role of aflatoxin in the illnesses of these persons. However, other Taiwanese investigators have supplied information to the present author strongly suggesting that moldy food is a factor in the relatively high incidence of liver cancer on Taiwan. Some cases that were diagnosed early were amenable to partial hepatectomy.

\(^1\)The singular "aflatoxin" is often used generically to include all of the closely related compounds implied in the plural of this word.
Peers and Linsell (35) divided Murang’a district of Kenya into three geographical areas based on altitudes (high, middle, and low) and analyzed for aflatoxin levels in foods prepared for human ingestion. The sampling continued over a period of 21 months to afford some measure of seasonal and annual replication. The data obtained were compared with national cancer registration data for this district provided by the Kenya Cancer Registry for the years 1967-1970. Diagnosis of hepatocellular cancer was based on histological findings, a positive alpha-foetoprotein test, or clinical diagnosis within 6 months of death when the first two criteria were not obtainable. A statistically significant association between ingested levels of aflatoxin and liver cancer cases in the designated altitude areas was obtained which required certain qualifications, because of possibly incomplete data, before the etiological significance was considered. There was an increasing frequency of both aflatoxin-contaminated diets and mean levels of food contamination as the area altitudes decreased which also corresponded with increased incidence of liver cancer. These workers cautioned that the Murang’a district findings covered only a small portion of possible associations of dietary aflatoxin and liver cancer cases and that additional studies should be done in areas with higher rates of liver disease or higher food levels of aflatoxin to test the strength and consistency of this association.

Swaziland

Primary liver cancer, particularly in males, has long been a serious problem in this small African country. Cancer Registry figures for 1964 to 1968 represent a crude rate of 8.6 per 100,000 for males and 1.6 for females. The incidence among the small number of Shangaan immigrants living in the same environment was considerably higher than the Swazis in the age group of 25 to 64 years. An attempt was made by Keen and Martin (26) to correlate the presence of aflatoxin in peanuts with the prevalence of primary hepatoma in four geographical divisions. The incidence of liver cancer decreased with increasing altitude; the risk of acquiring liver cancer in the lowveld was at least four times greater than in the highveld. Forty percent of all peanut samples collected contained aflatoxin, and the geographical sources of positive samples correlated well with the incidence of primary liver cancer in the different areas. The increased prevalence of hepatoma in the Shangaans was attributed to their peculiar habit of eating larger quantities of peanuts. Better harvesting and storage methods in the southern region of Swaziland apparently resulted in a smaller percentage of aflatoxin-positive peanut samples along with a lower number of primary liver cancers, as recorded by the Registry.

Peers et al. (36) extended their previous survey work in Kenya to Swaziland where three major and one minor altitude areas were selected for study. As before, “food from the plate” samples were obtained. The three major areas consisted of the highveld, middleveld, and lowveld regions mentioned above, whereas the Lebambe range in the extreme eastern part of the country made up the fourth area. This latter region approximates the middleveld in altitude but resembles the lowveld in respect to vegetation in many places. The primary liver cancer crude rates for all ages in Swaziland for 1964-1968 was 4.9/100,000 compared with 3.3/100,000 for all ages in the Murang’a district of Kenya for 1967-1970. Here, as in the Murang’a survey, the principal significant factor relating to aflatoxin contamination frequency and mean toxin levels was the altitude of respective areas in which food samples were collected. These workers found, as had Keen and Martin (26), that the northern parts of each region showed greater contamination incidence, but there was no significant difference in mean contamination levels between the north-south regions. Surprisingly, there was a lack of seasonal effect on the aflatoxin content of the diets. Peanuts, beans, and cultivated vegetables were included more frequently in the diets containing aflatoxins. Maize could not be specifically incriminated as a source of aflatoxin since it was included in almost all of the plate samples.

It was not possible from this study to show an increased risk for liver cancer among the Shangaans. Moreover, although there was an increased frequency of liver cancer in the northern portions of the high and middleveld areas, this was not true of the country as a whole. This study clearly demonstrated, however, that, at least in males, a logarithmic relationship existed between liver cancer incidence and aflatoxin ingestion.

Mozambique

According to Purchase and Goncalves (39), liver cancer is common among the relatively stable population of the Inhambane district of Mozambique. Based on 101 cases encountered in 1968 at the Chicuque Hospital, the liver cancer rate was 16 per 100,000 per annum with the male to female ratio 2.1 to 1. Maize, peanuts, rice, beans, manioc and cashew nuts were the staples drawn from nine families of liver cancer patients and from other family sources. Although the total number of food samples was small, there was a somewhat higher incidence of positive samples obtained from the families of cancer victims.

Van Rensburg and coworkers (59), in a 1974 report, gave additional data suggesting the Inhambane district had the highest known primary hepatoma rate in the world based on hospital registrations and data on occurrence of the disease from health records of gold miners in the study area. Their figures for the 1964-1968 period were 35.5 cases/100,000/year; and for 1968-1971, 25.4/100,000/year. The male to female ratio was 2 to 1. Aflatoxin assays on 880 meals, collected at random, revealed that 9.3% contained aflatoxin. The mean level of all prepared food was 7.8 mcg/kg wet weight, and the
mean daily per capita consumption was 222.5 ng/kg of body weight or 15.6 mcg/adult/day. These figures were compared with similar data from Kenya and Thailand. The study areas in Kenya had a hepatoma incidence of 3.2/100,000/year with an estimated intake of 7.8 ng/kg/day; and in Thailand, 7.3/100,000/year and 74 ng/kg/day. These pooled data indicated that over a wide range, cancer incidence appeared to be linearly related to the logarithm of the level of aflatoxin intake.

The Philippines

Campbell and Salamat (11) reported a survey carried out in 1967-1969 on aflatoxin contamination of a variety of foods including peanuts and peanut butter in the Philippines. They found that generally a better quality of peanut was selected for the sale of whole shelled kernels than was true for those used in peanut butter. The latter samples contained aflatoxin at a median value of 155 mcg/kg and a very high mean of 500 mcg/kg, whereas whole peanuts had a median of 17 mcg/kg. Candy containing peanuts was at an intermediate level of contamination. Highest concentrations of peanut aflatoxin were noted in the health region that included Manila (Region No. 3) where most of the locally produced peanut butter was consumed. Health region No. 6 included the island of Cebu, where maize was eaten by a large percentage of the people. This commodity was one of the more highly contaminated foods of that region. Both regions No. 3 and No. 6, also had the highest rates of liver cancer.

Twenty-four hour urine samples collected from individuals eating peanut butter with known concentrations of aflatoxin contained only the M1 toxin, presumably derived from B1 in the food. It was calculated that not more than 1 to 4% of ingested B1 appeared in the urine as M1, and that the minimum daily consumption of B1 required to produce detectable levels of M1 was 15 mcg/day. Human fecal and milk samples did not give positive results even when urine samples were positive for M1.

Southeast Asia

Shank and Wogan of MIT, along with various collaborators in Hong Kong and Thailand, carried out an extensive survey of fungus and aflatoxin contamination of prepared and market foods in Southeast Asia and compared the data with recorded incidence of liver carcinoma in various regions of that part of the world. In the initial phase (50) they screened more than 3000 food samples, showing that Aspergillus was the most common genus and that A. flavus was the predominant species occurring on foods. Penicillium, Fusarium, and Rhizopus were also frequently isolated. One hundred sixty-two isolates were obtained, and 49 of these produced toxins other than aflatoxins when grown on food materials. The extracts were fed orally to rats to determine toxicity.

Analysis of more than 2000 market foods and foodstuffs from Thailand (51), representing at least 170 different human foods, demonstrated that peanut products were the food items most frequently and highly contaminated with aflatoxins. Somewhat less often involved were dried corn, millet, wheat, barley, Job's tear seeds, and dried chilli peppers. The frequency and extent of contamination of foods followed geographical distributions and seasonal trends. In Hong Kong different varieties of beans constituted the chief sources of aflatoxins among the foodstuffs examined. The frequency and levels of aflatoxin in Hong Kong foods were lower than in Thailand. Rice in both places was seldom contaminated and only at low levels.

Three rural areas of Thailand were selected for sampling and analyzing prepared foods for aflatoxin (52). One hundred forty-four randomly selected households (families) in nine villages were the sources of cooked food collected by three 2-day surveys over a period of one year. Estimates of individual human intake based on the results of these analyses were highest in the Singburi and Ratburi areas with respective annual means of 73 to 81 ng and 45 to 77 ng total aflatoxin/kg/body weight/day. Intakes as high as 1072 ng/kg were noted for some individuals. In the Songkhla area the aflatoxin intake was 10 to 14 times less than in Singburi. People in two of these areas (high and low liver cancer areas) were surveyed for incidence of confirmed primary hepatoma. In line with data on aflatoxin ingestion obtained from Ratburi, the incidence of liver cancer was six new cases/100,000/year. In the town of Ratburi itself, the rate was 12.3 new cases/100,000/year. However, in the Songkhla area only two new cases/100,000/year were confirmed, which is comparable to the rates noted in parts of the United States. The male to female ratio overall in Thailand has been reported as 6 to 1 while in the U.S. the ratio was estimated at 1.5 to 1.

Senegal

Payet et al. (34) described the effects of inadvertent administration of aflatoxin-contaminated peanut meal in Kwashiorkor therapy to two African infants, just under 12 months of age, for nearly a year. Each received 70 to 140 g of peanut meal daily for an estimated intake of 35 to 140 mcg aflatoxin/day (5 to 20 mcg/kg of body weight). The meal contained 0.5 to 1.0 mg of aflatoxin/kg (type not specified). The children were located for study 4 and 6 years later. Biopsies revealed a fibrotic liver in one child which persisted through the sixth year; the other had minor histological changes in the liver which appeared to resolve within the next 2 years.

India

Robinson (41) in 1967 reviewed the epidemiology and possible causative agents of infantile cirrhosis and presented evidence strongly indicating that aflatoxin may be the prime factor. The syndrome was first described in 1887 and is now recognized all over India. Similar or identical conditions have been noted in Ceylon, Indonesia, West Africa, Costa Rica, Trinidad, Israel, Lebanon, Syria, Egypt, West Indies, Burma, and the
Soviet Republic of Tadzhekistan. The disease frequently strikes several children of the same family. Most cases in India have been in infants and children of 1 to 2½ years of age.

The initial histopathological change is seen as infiltration of hepatic cells with fat, followed by disintegration of centrolobular cells. Fibrosis soon ensues with enlargement of the liver, and yellow urine. In the following stage the spleen becomes enlarged, and the liver becomes shrunken. Jaundice becomes more pronounced as portal obstruction develops, producing ascites and edema of the limbs.

Analyses of the milk of Indian mothers of cirrhotic children were positive for aflatoxin B₁ in three cases out of 43 examined; 12 others exhibited violet fluorescent spots at other Rf levels on thin layer chromatography plates, and three gave fluorescent spots with colors other than violet. The remainder were negative. Eighteen of 50 urine samples from cirrhotic children were considered positive for B₁. Urines from normal subjects gave negative results.

Amla et al. (3) described the condition of 20 children (19 kwashiorkor, 1 nephritis), ranging in age from 1.5 to 5 years, who received daily food supplements of 30 to 60 g of peanut meal later found to contain 0.3 mg of aflatoxin B₁/kg. This would amount to 9 to 13 mcg toxin or an average of 1.1 mcg/kg of body weight. The meal was given for a period varying from 5 days to 1 month. Three children who received the supplement for 17 days developed signs of liver cirrhosis that began as a fatty liver and developed into cirrhosis over a one-year period. The characteristic clinical signs and histopathological lesions noted in biopsies were not seen before 6 months.

Sreenivasamurthy [cited by Campbell and Stoloff (47)] reported negative biopsy data for cirrhosis and negative clinical symptoms in kwashiorkor children receiving peanut meal containing 15 mcg/kg.

Krishnamachari and coworkers (29) described a 1974 outbreak of acute toxic hepatitis with high fatality rate in adult humans and dogs which was attributed to consumption of maize contaminated with aflatoxin. Analysis of the food indicated the affected persons, who were poorly nourished, may have eaten 2 to 6 mg of toxin daily for one month.

A total of 397 patients were studied in the adjacent states of Gujarat and Rajasthan. 106 of whom died. The contamination of grain was attributed to heavy rains in October and poor storage conditions. Death was usually sudden and in most instances was preceded by massive gastrointestinal bleeding. Males were affected twice as commonly as females. Dogs sharing the family diets were also stricken. Liver analyses did not show aflatoxin B₁ but did show other fluorescent spots. Of seven serum samples collected, two had detectable B₁ spots on thin layer plates, but all urine samples were negative. The outbreak of disease lasted only until the supply of moldy maize was exhausted. In afflicted families, persons who did not partake of the diet remained healthy.

Bile duct proliferation and periductal fibrosis were noted histologically. Jaundice, rapidly developing ascites, and portal hypertension were cardinal clinical signs.

The high levels of aflatoxin on the grain (6.5 to 15.6 ppm) would account for the massive gastrointestinal hemorrhaging which is similar to that recorded for dogs afflicted with hepatitis x (5) and experimental animals receiving high doses of aflatoxin orally (60).

**AFLATOXINS AND REYE'S SYNDROME**

Reye's syndrome, first described in 1963 as “encephalopathy and fatty degeneration of the viscera” (40) has since been recognized as a major cause of morbidity and mortality among infants and children (17). It is an acute disease that may affect those of a few months of age to adolescence. The disease often progresses from a mild prodromal viral illness with vomiting and abdominal pain due to influenza B or chickenpox, to cerebral involvement with coma. Blood ammonia levels are often high early in the course of the illness, whereas glucose levels are low. The mortality rate has been about 40%, but survivors may recover rapidly.

The Viral Diseases Division, Bureau of Epidemiology, at the Communicable Disease Center in Atlanta reported that 220 cases of Reye's syndrome were documented from 33 states and the District of Columbia for the period January 1 through March 31, 1977. In 30 of the cases, in which the outcome was known, 19 died, two survived with residual neurologic damage, and nine recovered completely.

One hundred thirty-nine cases of what appeared to be Reye's syndrome among children in Thailand were reported by Bourgeois et al (10) in 1969. Eighty percent of the hospitalized cases ended fatally. It has been estimated that several hundred children in Thailand, between the ages of 1 and 13 years, may die each year of this condition. The seasonal and geographic incidence of aflatoxins in Thai food markets seems to parallel frequency of the disease.

Aflatoxin analyses were reported by Shank and a group of Thai coworkers (49) on post-mortem specimens from 23 Thai children who died from acute encephalopathy and fatty degenerative changes in the visceral organs (EFDV), and from 15 children and adolescents who died of unrelated causes. Aflatoxin B₁ was found in one or more specimens from 22 of the 23 cases of EFDV. In two of the cases, very high concentrations of aflatoxin were found in liver specimens. A 2-year-old boy had 93 mcg of B₁/kg of liver, 123 mcg/kg in stomach and intestinal contents, and 8 mcg/ml of bile. Trace amounts of B₂ were demonstrated in post mortem specimens from 11 of the 15 control subjects.

Dvorackova et al. (18) found aflatoxin in the livers of two infants of three fatal cases presenting features of Reye's syndrome in Czechoslovakia. It was considered likely that the subjects were first exposed to aflatoxin during intrauterine life or soon after birth. Another group of investigators (14) detected what appeared to be...
an aflatoxin metabolite in the liver of a 15-year-old girl who died of Reye's syndrome. Seven other patients with the same diagnosis did not show chromatographic evidence of aflatoxins in their liver samples.

Hayes (24) detected B1, using high speed liquid chromatography, in the plasmas of three patients with Reye's syndrome recently at the University of Mississippi Medical Center. Concentrations of toxin ranged from 3.4 to about 12 ppb. Urine, liver, kidney, and brain samples were negative.

Harwig et al. (23) have reviewed various reports on Reye's syndrome and outlined the evidence supporting aflatoxin as an etiological factor. They called for submission of properly collected and transported liver specimens from suspected cases in Canada to the Health Protection Branch of the Canadian Health and Welfare Department.

It seems apparent that what is commonly diagnosed as Reye's syndrome could have more than one causative agent; i.e., a virus or a toxin, or possibly both agents acting in concert.

If the foregoing accounts are accurate in their implications, the evidence suggests that many thousands, if not millions, of people in different parts of the world may consume aflatoxins in several naturally contaminated foodstuffs. Children appear to be particularly susceptible and have, in several cases, been the victims of lethal quantities of toxin. The liver of man, as with primates and other animals, apparently is the principal target organ. Depending on the individual's age, levels of toxin intake, and the duration of dosing, the human response apparently may consist of acute to chronic hepatitis with clinical jaundice with fatty metamorphosis and necrosis of hepatocytes leading to death from internal hemorrhages. As in the case of chronic alcoholism, subacute to chronic intoxication from aflatoxin may cause liver cirrhosis. Also, as with other species, the human liver may show ductular cell proliferation and malignant neoplasia that ends in early death from the time hepatoma is first detected.

The importance of carefully planned human health surveys is evident as one studies data and conclusions from the foregoing reports. The necessity for compiling complete and accurate cancer statistics is matched by the need for valid analytical methods for aflatoxins in foods. Certain anomalous results, perhaps due to aflatoxin metabolites in human livers or urine specimens, need to be investigated in view of recently identified metabolic derivatives of these compounds. The possibility of false-positive data caused by unrelated substances from certain food sources should also be considered.

Finally, one must be aware of other means of acquiring poisoning from aflatoxins than through ingestion of contaminated foods. Those who work with these substances in the laboratory are prime subjects for accidental or careless exposure which can lead to skin contact, ingestion, and inhalation of toxins. Aerosols may be generated from mold dust, spills and pipetting of toxin solutions, scraping thin layer plates, and several other potentially hazardous procedures. Dvorackova (19) reported the observation of alveolar cell carcinoma in a 68-year-old chemical engineer who had worked for 3 months on methods of sterilizing bacterial cell carcinoma contaminated with A. flavus. Analysis of the excised lung demonstrated a blue fluorescent spot on thin layer chromatography whose Rf matched that from a commercially available sample of B1.

In areas where dairy products are a major food commodity for man the possible periodic contamination of milk with aflatoxins B1 or M1 should be considered since livestock are often fed aflatoxin-containing corn or other moldy grains. Such contamination of commercial milk with M1 was reported in South Africa by Purchase and coworkers (38) and in Germany (milk and cheese) by Kiermeir and coworkers (27,28). Processing of milk was shown to reduce the M1 content.

In the United States extensive regulation of food production and marketing by various governmental agencies tends to decrease chances of aflatoxin entering our food supply to a hazardous extent. Phillips and Yourtee (37), however, have recently reported finding B1 toxin in a liver biopsy specimen taken from a 56-year-old male Caucasian rural resident of Missouri who had cancer of the rectum and liver. It was estimated that 520 ng of B1 aflatoxin was present per gram of wet liver. This incident as well and the finding of B1 in Reye's syndrome patients suggests that more extensive surveys should be carried out to assess, if possible, the role of aflatoxins in human diseases in this country.

**DERMOTOXIC FUNGUS METABOLITES**

Mycotoxins known to produce dermal reactions in humans by direct contact are mainly of two sources. The first of these is pink-rotted celery infected with Sclerotinia sclerotiorum. Two compounds, 8-methoxyxpsoralen and 4,5,8-trimethoxyxpsoralen, produced by the fungus are capable of sensitizing the integument of celery harvesters with light skin so that subsequent exposure to light of about 320 to 400 nm wave length for a short period will give rise to bullous lesions (44). Photosensitization associated with food consumption by man has been reported but not well characterized (45). However, this syndrome is well known for animals that consume grass infected with Pithomyces chartarum and other naturally toxic plants. The photosensitization is thought to be caused by liver damage that results in accumulation of sensitizing serum and tissue levels of phylloerythrin, a metabolite of chlorophyll.

The second group of direct skin irritants belongs to the class of fungus metabolites known as 12,13-epoxytrichotheccenes. These compounds are produced by several food-contaminating fungi including species of Fusarium (6). Many also exert toxic effects on bone marrow and other tissues as mentioned earlier for the disease.
alimentary toxic aleukia. The author has known of two laboratory workers who suffered severe facial, upper limb, and trunk skin eruptions due to inadvertent direct skin contact with pure T-2 toxin or culture extracts of *F. graminearum*. It is quite clear why inhalation of trichothecene-containing fungus dust from grain, hay, or other contaminated plant material can produce upper respiratory tract irritation in humans as reported by Russian investigators for hay infected with *Stachybotrys atra (alternans)* (9).

**HUMAN DISEASES WHICH MAY BE ATTRIBUTABLE TO FUNGUS TOXINS**

**Neurological diseases**

There are now several tremorgenic mycotoxins recognized which are produced by food-contaminating fungi. The complex chemical structures of certain members have also been elucidated (15). It seems reasonable to suppose that these substances may be finding their way into human foods on occasion. Although they are potent neurotoxins, producing acute reactions in animals at dosages of a few milligrams/kg of body weight, it probably would require ingestion of a considerable amount of well-molded food at one sitting to produce noticeable effects in man.

In western Nigeria a seasonal affliction of natives, known as Ijesha Shakes, is a fairly common diagnosis upon hospital admission (63). The cause is unknown, although cyanogenic glycosides or tremorgen-containing foods are considered likely possibilities. Symptoms of the disease usually begin after eating, and nearly complete incapacitation is caused by intentional tremors, particularly of the legs, when the patient attempts to stand. The trembling may last for a few days followed by complete recovery. Certain outbreaks of “grass staggers” of sheep and cattle in New Zealand appear to be identical to the neurological disease signs induced by feeding pure toxin or mycelium of *Penicillium cyclopium* which contains the well-known tremorgenic toxin called penitrem A (61).

**Balkan nephropathy**

Balkan nephropathy is an endemic disease of people living in close proximity to the Danube River and its tributaries in Yugoslavia, Romania, and Bulgaria (32). It is estimated that there may be more than 20,000 cases within rural areas of these regions. The disease presents a clinical picture of slowly progressing renal failure, seldom causing sodium retention or systemic hypertension. The cause(s) of the disease remains unknown in spite of broad studies attempting to associate it with several possible causative agents. The possibility of a mycotoxin etiology has been attractive, and studies to ascertain the incidence of ochratoxin in foodstuffs used in the Balkan region have been made by at least two groups. Krogh (30) has indicated that 6 to 20% of the foodstuffs in one endemic village in Yugoslavia contained ochratoxin A, a well known nephrotoxic substance for swine and poultry in Scandinavia (31).

However, the late J. M. Barnes and five coworkers recently published a report (8) showing that *Penicillium verrucosum var. cyclopium* was the most frequent isolate from some 163 samples of foodstuff and other materials collected from five endemic areas of the disease. Liquid cultures of one of the isolates was force-fed to young rats and caused subtle histological changes in cells of the lower part of the proximal convoluted tubules. The location of the lesions and their morphology were considered to be almost identical to those in the disease of man. Speculation that the toxic principle(s) may be the cause of Balkan nephropathy will certainly lead to additional study.

This review has mentioned only a few of the many recognized mycotoxins that could be involved in human health. Undoubtedly, several other important metabolites remain undiscovered or unrecognized as toxins. A pressing need exists for suitable analytical methods permitting detection and quantitation of the more nontremorgenic mycotoxins that may occur in human foods. Recommendations for further research on this and related problems were generated by a meeting of the United States-Japan Conference on Mycotoxins in Human and Animal Health held at College Park, Maryland in October, 1976. Continued research and vigilance on the part of governmental regulatory agencies concerning mycotoxins are required to afford protection to human and animal population.

**REFERENCES**


