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# Dietary Factors and Special Epidemiological Situations of Liver Cancer in Thailand and Africa[1]

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**Summary**

Incidence patterns of primary liver cancer in Swaziland and Uganda have been compared with frequency of contamination of dietary staples by aflatoxins. Geographical regions or tribal groups with elevated cancer incidence were associated with increased frequency of contamination. In further studies, aflatoxin ingestion has been quantitatively measured in populations in Thailand, Kenya, and Mozambique, in subgroups of which the incidence of primary liver cancer varied over a wide range. In each instance, elevated cancer incidence was associated with highest levels of aflatoxin intake. In view of the potency of these compounds as liver carcinogens in many animal species, these data collectively suggest that the aflatoxins are also carcinogenic for man and that regular ingestion of foods heavily contaminated with aflatoxins increases the risk of liver cancer in human populations.

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Direct information on the responsiveness of humans to chemicals known to be carcinogenic to animals is lacking except in a very few instances. Evaluation of the importance of chemical agents in the induction of human cancers therefore currently depends on assessment of exposure to specific carcinogens in populations with different disease incidence. Existing information of this kind has come mainly from exposures in industrial or occupational settings.

Aflatoxins are among the few chemically identified and widely disseminated environmental carcinogens for which quantitative estimates of human exposure have been systematically sought. Despite the fact that significant differences in responsiveness are known to exist among animal species, it is reasonable to assume that man might respond to either acute or chronic effects of the toxins in the event that exposure takes place through contamination of dietary components. It also seems reasonable to assume that the character and intensity of the human response might vary depending upon factors such as age, sex, nutritional status, concurrent exposure to other agents (*e.g.*, herbal medicines), genetic factors, concurrent illness (*e.g.*, viral hepatitis or parasitic infestation), as well as level and duration of exposure to aflatoxins.

As information has accumulated on various aspects of the aflatoxin problem (3), it has become evident that the risk of exposure to aflatoxins is much less in technologically developed countries than in developing areas. The lower risk is attributable to the combined effects of several factors contributing to prevention of contamination of foods or food raw materials. The use of such agricultural practices as rapid postharvest drying of crops and controlled storage conditions tends to reduce mold damage in general and thereby also reduces the likelihood of aflatoxin contamination.

In societies not equipped technologically to apply such practices, the risk of aflatoxin exposure is clearly much greater. Since their discovery, reports have occasionally been made of the identification of aflatoxins in many kinds of human foods collected in various parts of the world (2, 10). Although these findings indicated the widespread geographic nature of the problem, they provided little useful information on human exposure, since samples were randomly collected and it was unknown whether they would actually have been eaten.

Information has recently become available from studies carried out by several groups of investigators in different countries of Africa and Asia. All of these investigations were designed to obtain estimates of aflatoxin intake by populations in which primary carcinoma of the liver occurs at different incidences. The pertinent findings of these investigations can be briefly summarized in the following way.

Keen and Martin (4) analyzed for aflatoxins market samples of peanuts collected in various localities within Swaziland. They attempted to estimate aflatoxin intake by interviewing the consumers concerning habitual patterns of peanut ingestion. Data obtained are summarized in Table 1. In the highveld region, 20% of the peanut samples contained detectable levels of aflatoxins, whereas those from the middleveld and lowveld regions were contaminated at rates of 57 and 60%, respectively. The geographical distribution of liver cancer in Swaziland, derived from cancer registry data, are also summarized in Table 1, and the authors suggest a higher disease risk in those areas with highest frequency of aflatoxin contamination. These data provide only a general impression of a pattern of potential exposure, since measurements were made on only 1 dietary component and intake was not measured. The data are, however, suggestive enough to warrant further investigation.

A study of somewhat similar design was conducted in Uganda (1), and the results are summarized in Table 2. In this instance, an attempt was made to collect samples of all

[1] Presented at the Conference on Nutrition in the Causation of Cancer, May 19 to 22, 1975, Key Biscayne, Fla.

Table 1

*Geographical distribution of malignant hepatoma in Swaziland 1964 to 1968*[a]

| | No. of cases | Crude rate/ $10^5$/yr | Population ratios | Relative risk | Corresponding aflatoxin assays in groundnuts (% positive samples) |
|---|---|---|---|---|---|
| Highveld | 11 | 2.2 | 1.0 | 1.0 | 20 |
| Middleveld | 34 | 4.0 | 1.7 | 1.8 | 57 |
| Lowveld | 44 | 9.7 | 0.9 | 4.5 | 60 |

[a] Keen and Martin (4).

Table 2

*Aflatoxin contamination of foods vs. hepatoma incidence in Ugandan tribes*[a]

| | Hepatoma incidence (cases/$10^5$/yr) | Aflatoxin-containing foods (%) | No. of samples |
|---|---|---|---|
| Bwamba | No data | 79 | 29 |
| Karamojong | 15.0 | 44 | 105 |
| Baganda | 2.0 | 29 | 149 |
| West Nile | 2.7 | 23 | 26 |
| Acholi | 2.7 | 15 | 26 |
| Soga | 2.4 | 10 | 39 |
| Nakole | 1.4 | 11 | 37 |

[a] Alpert *et al.* (1).

major diet ingredients from home granaries. In all, a total of 480 food samples were analyzed during 1 year (1966 to 1967). Among these samples, 29.6% contained detectable amounts of aflatoxins, and 3.7% contained more than 1 ppm. Most frequently contaminated diet ingredients were beans, maize, and sorghum, although most staples were contaminated to some extent. The data in Table 2 are arranged in order of decreasing frequency of aflatoxin contamination, and include tribal distribution of hepatoma incidence as determined by identification of new cases. Highest incidence of the disease was recorded in tribal regions in which frequency of aflatoxin contamination was also high (44% of samples). Although these data provide a somewhat clearer indication of patterns of aflatoxin intake among the tribal groups involved, accurate values for actual intakes cannot be calculated since food intake measurements were not made and cooked foods were not analyzed.

Peers and Linsell (5) have investigated aflatoxin ingestion and hepatoma incidence in Kenya, with the results summarized in Table 3. Aliquots of total diet (as eaten) and of beers (as consumed) were systematically collected and analyzed for aflatoxins. In 3 subareas circumscribed on the basis of altitude, the frequency of contamination varied from 4.8 to 9.5% in diet samples and from 3 to 9% in beer samples. Average aflatoxin intakes were calculated on the basis of assumed daily intakes of food and beverages. As in previous studies, the groups with higher average aflatoxin intakes also had elevated incidence of hepatoma.

A study with similar general objectives was previously completed in Thailand (6–8). The investigation was conducted in 3 phases, the first being a survey of market samples of foods. Over a 23-month period, 2180 samples of more than 170 varieties of foods and foodstuffs were collected from markets, mills, warehouses, distributors, farms, and homes throughout Thailand. A total of 204 (9%) of these samples were contaminated with aflatoxin (Table 4). Peanuts and corn were the most frequently contaminated foodstuffs (49 and 35% of the samples were contaminated, respectively) while 11% of the millet and dried chili pepper samples contained aflatoxins. Total aflatoxin concentrations in what superficially seemed to be wholesome foods or foodstuffs destined for human consumption were as high as 772 ppb in dried fish, 966 ppb in dried chili peppers, 2.7 ppm in corn, and more than 12 ppm in peanuts.

Such extensive contamination of market foods and foodstuffs was used to design a dietary survey to measure more accurately the amounts of aflatoxins actually consumed. On the basis of results from the market study, 3 areas in Thailand representing suspected high, intermediate, and low levels of contamination were chosen for such a dietary survey. Three representative villages were selected in each area, and 16 families in each village, randomly chosen, constituted the survey population (Table 5). A portion of the diet of each family was collected and assayed for aflatoxins for 3 separate 2-day intervals over a period of 1 year. Samples of as many prepared foods as was feasible were collected, and a sample of cooked rice was obtained from almost every meal of each family. In most cases, 30 to 50% of the prepared foods in the diet were analyzed. As shown in Table 5, the proportion of contaminated samples was lowest in Songkhla (1%) and highest in Ratburi (16%). Similarly, levels of contamination were lowest in Songkhla (only 1 sample containing more than 50 ppb) and higher in the other regions.

Based on weights of cooked foods eaten at each meal, aflatoxin intake was calculated, on a family basis, for each region (Table 5). In the Singburi area, an average of 73 to 81 ng total aflatoxins per kg body weight on a family basis were consumed each day. In the 2nd area, Ratburi, the average was 45 to 77 ng per kg body weight per day; in Songkhla (the area of lowest contamination), the average was 5 to 8 ng per kg body weight per day. In a few instances, it was possible to measure the amount of aflatoxin consumed in 1 day by individual members of survey families. The highest single daily consumption measured was for a 75-year-old woman who ingested 1072 ng total aflatoxins per kg body weight. The contaminated food in this case was cooked rice, probably left over and resteamed several times.

The most heavily contaminated foods were a dish containing cabbage fried with pork and garlic (1.3 ppm total aflatoxins), sun-dried fish (0.8 ppm total aflatoxins), and a dish containing fresh shrimp fried with pork, garlic, and chili peppers (0.4 ppm total aflatoxins). While it would not be anticipated that these foods (none of which contained peanuts) would be among the most heavily contaminated dishes, the data are consistent with the results from the market study indicating significant contamination in garlic, dried chili peppers, and dried fish, all common ingredients in Thai foods.

Table 3

*Daily aflatoxin intake and liver cancer incidence in Kenya*[a]

Data for males ≥16 years old.

| Altitude sub-area | Diet contamination | | Beer contamination | | Mean aflatoxin ingested[b] (ng/kg body wt/day) | Liver cancer incidence |
|---|---|---|---|---|---|---|
| | Frequency | Mean aflatoxin level (ppb) | Frequency | Mean aflatoxin level (ppb) | | |
| High | 39/808 | 0.121 | 3/101 | 0.050 | 4.88 | 3.11 |
| Middle | 54/808 | 0.205 | 4/101 | 0.069 | 7.84 | 10.80 |
| Low | 78/816 | 0.351 | 9/102 | 0.167 | 14.81 | 12.12 |

[a] Peers and Linsell (5).

[b] Assuming intake of 2 kg of food and 2 liters of beer per day per 70 kg.

Table 4

*Aflatoxin contamination of selected Thai foods*[a]

Market samples 1967 to 1969.

| Foodstuffs | % contaminated | Aflatoxin mean | Content (ppb) maximum |
|---|---|---|---|
| Peanuts | 49 | 1,530 | 12,256 |
| Corn | 35 | 400 | 2,730 |
| Chili peppers | 11 | 125 | 966 |
| Millet | 11 | 67 | 248 |
| Dried fish | 5 | 166 | 772 |
| Mung beans | 5 | 16 | 112 |
| Rice | 2 | 20 | 98 |

[a] Shank *et al.* (7).

Peanuts were found not to be common to many prepared foods in Thailand. Indeed, peanuts and peanut products were usually eaten as snacks away from home, and it was not possible to determine aflatoxin intakes from peanut sources for this reason. However, several observations were made that are of interest to this discussion. For example, children consumed peanuts more frequently and in larger quantity on a body weight basis than adults. Children aged about 3 years, weighing 10 to 12 kg, were observed to eat up to 250 g of shelled boiled peanuts in 1 day during harvest season. The aflatoxin $B_1$ content of boiled peanuts has been as high as 6.5 ppm; thus, a 10-kg child could consume as much as 163 μg aflatoxin $B_1$ per kg body weight in 1 day by eating 250 g of such highly contaminated peanuts.

Although the absolute values of aflatoxin ingestion appear to be quantitatively small, the potency of these compounds as carcinogens in animals must be kept in mind in order to put these data into perspective. The highest values in Singburi, based on the yearly average total aflatoxin consumption, amount to 20 to 30% of comparable intakes that induce nearly 100% tumor incidence in rats following continuous exposure. Also, because these are family averages, exposures to individual family members are undoubtedly higher.

Included in Table 6 are incidence values for liver cancer in the lowest and intermediate aflatoxin intake regions. These values, calculated from new cases identified during the 3rd phase of the study, showed a 3-fold difference between the low- and intermediate-intake regions, being higher in Ratburi than in Songkhla. Unfortunately, incidence in the 3rd district was not determined.

Table 5

*Aflatoxin contamination of Thai foods*[a]

Cooked samples in 3 regions.

| | No. of foods or samples | | |
|---|---|---|---|
| | Singburi district | Ratburi district | Songkhla district |
| Foods eaten | 2640 | 2943 | 2008 |
| Samples assayed | 1021 | 1005 | 922 |
| Samples contaminated | 45 | 159 | 11 |
| Aflatoxin content (ppb) | | | |
| Trace | 22 | 129 | 10 |
| <50 | 4 | 19 | 0 |
| 50-100 | 10 | 7 | 1 |
| 100-200 | 3 | 3 | 0 |
| >200 | 6 | 1 | 0 |

[a] Shank *et al.* (8).

Table 6

*Daily aflatoxin intake and liver cancer incidence in Thailand*[a]

Data for both sexes ≥16 years old.

| Region | Mean aflatoxin intake (ng/kg body wt/family) | Liver cancer incidence (new cases/$10^5$/yr) |
|---|---|---|
| Songkhla | 5-8 | 2 |
| Ratburi | 45-77 | 6 |
| Singburi | 73-81 | |

[a] Shank *et al.* (6).

The relationship between aflatoxin intake and liver cancer incidence has recently been examined in the highest known incidence area of the world, namely the Inhambane district of Mozambique (9). Based upon a hospital registration program and health records of gold miners originating from the study area, the incidence of liver carcinoma for the period 1969 to 1971 was calculated as 25.4/100,000/year.

The extent of aflatoxin contamination of prepared foods consumed by the study population was determined by chemical assay of 880 meals collected at random. Aflatoxin was found in 9.3% of all samples, with a mean concentration of 7.8 μg/kg food (ppb). The mean daily per capita ingestion of aflatoxins was calculated to be 222.4 ng/kg body weight (15 μg/adult/day).

Pooling of these observations with the data from similar studies in other regions reveals a significant correlation between the level of aflatoxin consumption and liver cancer

Table 7

*Summary of current evidence on aflatoxin intake and liver cancer incidence*

| Population | Aflatoxin intake (ng/kg body wt/day) | Liver cancer incidence (cases/$10^5$/yr) |
|---|---|---|
| Kenya (high altitude) | 4.88 | 3.11 |
| Thailand (Songkhla) | 5.00 | 2.00 |
| Kenya (Middle altitude) | 7.84 | 10.80 |
| Kenya (low altitude) | 14.81 | 12.12 |
| Thailand (Ratburi) | 45.00 | 6.0 |
| Mozambique (Inhambane) | 222.40 | 25.4 |

incidence (Table 7). This evidence cannot be regarded to constitute unequivocal proof that aflatoxins are the single cause of liver cell carcinoma in man. However, the data are sufficient to suggest that exposure to these carcinogens substantially elevates risk of this disease, and they therefore warrant continued investigations into effective means for monitoring and control of the appearance of aflatoxins as food contaminants.

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