Nonassociation of Aflatoxin with Primary Liver Cancer in a Cross-Sectional Ecological Survey in the People’s Republic of China

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ABSTRACT

A comprehensive cross-sectional survey was undertaken in The People’s Republic of China of possible risk factors for primary liver cancer (PLC) to include 48 survey sites, an approximately 600-fold aflatoxin exposure range, a 39-fold range of PLC mortality rates, a 28-fold range of hepatitis B virus surface antigen (HBsAg+) carrier prevalence, and estimation of exposures for a large number of other nutritional, dietary, and life-style features.

PLC mortality was unrelated to aflatoxin intake (r = 0.17) but was positively correlated with HBsAg+ prevalence (P < 0.001), plasma cholesterol (P < 0.01), frequency of liquor consumption (P < 0.01), and mean daily intake of cadmium from foods of plant origin (P < 0.01).

Multiple regression analyses for various combinations of risk factors showed that aflatoxin exposure consistently remained unassociated with PLC mortality regardless of variable adjustment. In contrast, associations of PLC mortality with HBsAg+, plasma cholesterol, and cadmium intake remained, regardless of model specification, while the association with liquor consumption was markedly attenuated (was nonsignificant) with adjustment for plasma cholesterol.

The sharp contrast between the findings of no aflatoxin effect upon PLC prevalence in this survey and the positive correlation reported for previous but more restricted surveys is discussed. Based on the results of this survey and the data of laboratory animal and in vitro studies, an explanatory model for the etiology of PLC is proposed, taking into consideration the role of nutrition in the etiology of this disease.

INTRODUCTION

Aflatoxin is a group of difuranocoumarin metabolites of Aspergillus flavus that are exceedingly carcinogenic for selected species of experimental animals, mostly producing hepatocellular carcinoma (1–3). The most carcinogenic member of this group is aflatoxin B1, which also is the most commonly found. Accordingly, the association of aflatoxin with PLC in humans has been investigated in numerous populations, particularly in Southeast Asia and Sub-Saharan Africa (4, 5).

Evidence of aflatoxin cancer risk for humans periodically has been reviewed by expert committees of various institutions including the IARC (4–6) and the World Health Organization (7), among other agencies. The accumulation and interpretation of this evidence has gradually produced a consensus, particularly in the marketplace (8), that aflatoxin is a significant human carcinogen. According to IARC criteria (5) a conclusion of “sufficient evidence” means that “a positive relationship has been observed between exposure to the agent and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.”

It is not clear, however, that there is reasonable agreement on this new certainty (9–13). Some of the lingering skepticism is due to the failure of the early aflatoxin studies to take account of the almost certain and later discovered risk contributed by persistent infection with hepatitis B virus (that is, for those individuals who carry the viral surface antigen, HBsAg+) (14–16). Thus, when several recent surveys (17–19) reported an aflatoxin effect independent of HBsAg+ prevalence, considerable justification undoubtedly was given for the 1987 IARC report to change the strength of the evidence from “circumstantial” (4) to “sufficient” (5). However, the results of these three studies hardly justified this important change. The first of these studies, a communication in 1984 by Sun and Chu (17), did not produce comparative data on aflatoxin and HBsAg+ prevalence as implied by the IARC report (5). Instead, these investigators only speculated on preliminary methodological data from their laboratory and commented on other reports of two small localities in China (20, 21) where there was poor correspondence between HBsAg+ and PLC. No aflatoxin intake data were given in the Sun and Chu (17) report. The second communication (18), published in 1985, was limited to a cross-sectional comparison of two villages in the Guangxi Autonomous Region in southern China, with one “high” and one “low” in aflatoxin intake. The third cross-sectional study (19), reported in 1987, was a reinvestigation by the same investigators of four survey regions in Swaziland studied earlier (22), although these four regions were subdivided into 10 smaller areas and HBsAg+ prevalence also was measured. Aflatoxin intakes of 48–269 ng/kg/day were only modestly higher than intakes of 13–197 ng/kg/day estimated for southeastern regions of the United States (23) where no relationship with PLC was observed. The authors of the 1987 Swaziland study also pointed out that aflatoxin intake estimates were crude because the consumption of peanuts, “... the most heavily contaminated food item...” was not weighed at the time of collection (Intake was estimated indirectly by noting the frequency of peanut use in sauces). In addition, PLC mortality rates per 100,000 persons (5.8 for males and 1.8 for females in 1981) were surprisingly low, particularly in view of the very high prevalence (20–35%) for the 10 survey regions) of HBsAg+ carriers (19). Further calculation of these data indicate that only about 1.8% of the carriers may be expected to die of PLC, compared with 40–50% in...
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Taiwan (16). These anomalies detract from the conclusion of an independent aflatoxin risk.

Two other studies were also cited by the 1987 IARC report (5) as providing evidence for an aflatoxin effect on PLC. One study (24), which furnished "additional evidence for a causative association between aflatoxin and human cancer" (5), was a retrospective cohort study of 71 individuals who had been exposed as workers to variable amounts of aflatoxin dust in an oilseed extraction plant (24). Although increases in mortality rates for total and respiratory cancer were noted, these increases were not statistically significant and no cases of PLC were reported. A second case-control study in the Philippines (25) produced an aflatoxin effect but, again, no data were available concerning the prevalence of HBsAg+. Also, in this study, no confirmatory analysis was indicated for the fluorescent material suspected of being aflatoxin B1; one-half of the total intake was present in cassava, a source likely to be only occasionally contaminated, and confirmation of aflatoxin B1 identity is required.

Since the 1987 IARC report (5) was released, two additional cross-sectional studies (12, 26) have been reported. When four communes in southern China were compared (26), a linear relationship was observed for mean annual per capita consumption of aflatoxin during 1978-1984 and PLC mortality rates for 1982-1986. Aflatoxin intake (population-based rather than personal assessment) ranged 170-fold (17-2896 ng/kg/day, based on an assumption of 49 kg average body weight measured for adult males in the same area by Chen et al. (27)), while the range of PLC mortality rates was 3.5-fold (175-614/100,000 person years, 25- to 64-year-old men). In the cross-sectional comparison of these four communes, prevalence of HBsAg+ was not associated with PLC, although, in a case-control study of individuals within this same cohort, a relative risk of 38.6 was observed for HBsAg+ carriers when compared to noncarriers. In the second report, a cross-sectional study of 8 districts in Kenya (no aflatoxin data were available for one site), Auttrop et al. (12) examined the association of PLC incidence with HBV prevalence (HBsAg+ and antibody to HBV core protein) and aflatoxin exposure, as measured by prevalence of individuals who excrete in their urine a major aflatoxin-DNA adduct excision product. No association between HBV and PLC was observed. When all areas and ethnic groups were considered, no association between aflatoxin exposure and PLC incidence was found, but when the analysis was limited to the Bantu groups a "reasonable correlation" was obtained, according to the authors. However, no data were provided on the proportion of Bantu people in the total population. A comparison of the PLC prevalence rate between the study of Yeh et al. (26) and Auttrop et al. (12) reveals a substantial difference of perhaps 2 orders of magnitude in prevalence rates between these two areas, after adjustment for sex and age distributions and assuming incidence and mortality rates to be similar.

On the basis of these human population studies, particularly those cited by the 1987 IARC report (5), it is reasonable to remain skeptical of the suggestion that there is "sufficient" evidence for significant aflatoxin cancer risk in humans. The uncertainty of the evidence that aflatoxin is a significant PLC risk factor prompted interpretation of relevant portions of data obtained from 48 counties in a comprehensive survey of dietary, life-style, and mortality characteristics in 65 rural counties in the People's Republic of China undertaken by Chen et al. (27).

These data were uniquely comprehensive and broad ranging when compared with previous studies of aflatoxin and PLC. A companion interpretation of additional variables from this same data set (27) is being prepared by Drs. Ann Hsing and William Blot at the National Cancer Institute in the United States and will be published elsewhere.

MATERIALS AND METHODS

The study design for the complete cross-sectional ecological survey data used in this analysis was described by Chen et al. (27). Only those details relevant to the data presented in this communication are presented below.

Study Design. A multistage sampling procedure, more fully described by Chen et al. (27), was used to select survey counties. For the original survey, 65 counties were selected from a total of 2392 in order to obtain a wide geographic scatter and to encompass the full nationwide range of mortality rates for 7 of the more common cancers (nasopharynx, esophagus, stomach, liver, colorectal, lung, and leukemia). Two communes were randomly selected within each county and similarly within each commune, one production brigade, and within each production brigade two production teams were randomly chosen, yielding a total of 130 communes and 260 production teams. However, the survey communes were restricted to those within 4-5 of the originally selected communes. As described by Chen et al. (27), within each of the 260 production teams, 25 households were randomly selected from an official registry of residences (100 residences/county, 50 households/ commune). From each household either one male or one female aged 35-64 years, randomly selected, was then invited to donate blood, with an approximately equal number of individuals for each sex and age group: 35-44, 45-54, and 55-64 years. "If the selected subject in each household was absent or declined to participate (<1% of those initially identified), then another subject in a neighboring household was selected. The total number of participating subjects aged 35-64 years was 6500."

Because mortality data were available for 1973-1975 and biochemical data were collected in 1983, any analysis of the relationship between these biochemical parameters and mortality data is valid only if the biochemical indicators measure stable characteristics which have not changed much over time. Evidence for the stability of these characteristics is indicated by the negligible migration in this population (an average of 94% of the survey subjects still lived in the county in which they were born) and the likelihood that dietary patterns at the time of the survey reflected past dietary patterns which have been dependent on stable, locally available crops. Time trend data of national food intake show that dietary composition, for example, including grain (corn) intake (an indicator of aflatoxin exposure), has remained remarkably constant from 1950 until very recently (28). Also, a review of aflatoxin contamination data for a county in the Guangxi Autonomous Region where a good monitoring procedure has been in place since 1972 revealed a mean annual contamination rate of 69.6 ± 15.3% (SD) positivity (range, 41-88%) for the years of 1972-1985 (data made available to the Institute of Nutrition and Food Hygiene in Beijing).

Mortality Rates. PLC mortality rates were determined retrospectively for the years 1973-1975 and were published originally as the Atlas of Cancer Mortality (29, 30). Details of data collection, methods, and disease diagnosis and classification procedures were presented by Li et al. (30). The reliability of these data were affirmed on the basis of several criteria. First, 96% of the PLC deaths, as recorded, were attended by medical staff either at commune clinics (with one or more fully qualified physicians) or at county, prefecture, or province hospitals (including full medical services such as pathology, radiology, etc.). More important, 69% of liver cancer deaths had an antemortem diagnosis based on the analysis of pathological or histological materials (9%) or on gross assessment of specimens, operation, X-ray, ultrasonography, isotope microassay, biochemical immunology, or the like (60%). Also, care was taken to distinguish causes of death easily confused with one another, such as liver cancer and hepatocirrhosis. Second, disease rates for counties juxtaposed on opposite sides of provincial borders and
surveyed by different teams were similar. Third, diagnostic errors were very small when compared with the nationwide range of mortality rates. Fourth, a repeat survey in the Guangxi Autonomous Region (18) in 1987 showed a liver cancer rate of 94/100,000 persons compared with a rate of 96/100,000 persons obtained in the 1973–1975 survey for the same county group.

In addition, it should be noted that any analysis of the relationship between the mortality data of 1973–1975 and the dietary and ecological data of 1983 is valid only if stable characteristics not changing much over time were measured. Stability of the 1983 dietary characteristics was suggested by negligible migration of the subjects (an average of 94% of the survey subjects still lived in the county of their birth) and the likelihood that the dietary patterns at the time of the survey reflected past dietary patterns which have been dependent on stable, locally available crops. A current survey of causes of death for the years 1986–1988 coded according to the ninth revision of the International Classification of Diseases is presently being undertaken and these data will prove to be very useful for comparison.

Blood Collection. Ten ml of blood was obtained from each of the adults between 6 a.m. and noon, using trace mineral-free Vacutainers (Becton-Dickinson) Inc., Rutherford, NH). Samples were placed on ice and centrifuged (2000 rpm, 10 min) into RBCs and plasma. For each individual subject, one 4 ml fraction of plasma, containing 20 mg of sodium ascorbate, was then saved at −15°C to −20°C. Blood samples were collected from September to December 1983.

Urine Collection. Only 51 of the 65 survey counties provided urine samples (i.e., 4-h collections) considered to be adequate for this project (the remaining 14 counties only provided overnight collections which were not appropriate for this analysis). In each of these counties, about 25 males aged 35–64 years and residing in the same commune of each county gave 4-h urine samples between 6:00 am-12:00 noon after they had been given, as part of an accessory investigation, an oral ‘load’ of riboflavin and ascorbic acid to measure the excretion of these vitamins. Samples were shipped from the survey sites to the nearby county laboratories within 4 h of collection of the last sample. A 5% aliquot of each sample was then added to age-specific county pools (35–44, 45–54, 55–64 years) which were stored at −15°C to −20°C. Within 1–2 months all samples were shipped on dry ice to the Institute of Nutrition and Food Hygiene laboratories in Beijing where they were stored at −30°C until age-specific pools were prepared for other assays not reported here. On February 21, 1984, the pools were shipped to Cornell University in Ithaca, NY, and were stored at −80°C. During June 1985, samples were thawed and divided into subsamples of various size for various assays and stored at −80°C.

Aflatoxin Analyses. Only 48 of the 51 counties providing urine samples were analyzed for aflatoxin metabolites because samples from 3 counties were lost at the time of the analyses. The possibility of the introduction of an unintended selection bias was considered and the necessary omission of 17 of 65 (26%) of the sampling units was judged to have no serious statistical impact. First, the full range of the outcome variable (PLC rates) was maintained (50% of the omitted counties were above the median PLC rate) and, second, the loss of these 17 data points is not to have any unfavorable statistical effect when using the corn ration as a potential indicator of aflatoxin exposure (8 of 17 counties being above the median corn ration for all 65 counties). These aflatoxin analyses were undertaken in the laboratories of Dr. John Groopman at Boston University according to modifications of previously published methods (31–33). Each age-specific pool was analyzed for aflatoxin metabolites and then converted to weighted averages for each county, depending on the number of individuals in each age group. Before analysis, each sample was cleaned by passing 25 ml through a C18 Sep-Pak and isolating the 80% methanol eluant which was then dried and redissolved in 4 ml of water. This procedure isolates the lipophilic compounds in the urine, including the aflatoxins. To obtain the oxidative aflatoxin metabolites, the water fraction was extracted with 3 volumes of ethyl acetate which was dried in vacuo and redissolved in 4 ml of water. This water sample was applied to an anti-aflatoxin monoclonal antibody affinity column, which was subsequently washed with 3 volumes of phosphate-buffered saline, and then eluted with 6 ml of 50% dimethyl sulfoxide to produce an eluant containing the purified aflatoxins. After concentration under reduced pressure to 500 μl with a rotary evaporator, a 100-μl aliquot was analyzed by a competitive 1H-base radioimmunoassay. For each assay, 400 μl total volume was routinely used [100 μl of which consisted of the ['H]aflatoxin B, tracer (specific activity, 24 Ci/mmoll], representing a dilution in 1% normal mouse serum/0.1% bovine serum albumin in phosphate-buffered saline to a level of about 10,000 cpm/100 μl. The monoclonal antibody was diluted to a concentration which precipitated 40–60% of the aflatoxin B, tracer. The antibody was added to the reaction mixture in 100 μl of 1% bovine serum in phosphate-buffered saline. The test sample, either cold aflatoxin B, (for standard curves) or antibody column eluant reactive material, was added to 200 μl of a phosphate-buffered saline mixture. The reaction was incubated for 2 h at ambient temperature. An equal volume of ice-cold saturated ammonium sulfate was added to the reaction vessel, and the antibody was allowed to precipitate for 15 min. The precipitate was centrifuged for 15 min at 12,000 × g, and 400 μl of the supernatant was counted with an LKB model 1211 beta counter to quantify the amount of antibody inhibition.

Plasma Hepatitis B Virus Assays. Individual samples were used for the determination of hepatitis B virus indicators and data are expressed as prevalence of positive samples. Only plasma HBsAg data are given in this communication and these analyses were undertaken on individual samples (50 individuals in each of 48 counties) in the laboratory of Dr. Chongbo Liu using a SPRIA diagnostic kit (34). Plasma levels of hepatitis B virus anti-core antibody also were determined on individual samples by the enzyme-linked immunosorbent assay of Engvall and Perlman (35).

Biochemical Assays. Plasma total cholesterol was analyzed by the method of Parekh and Jung (36) according to the modification of Feng et al. (37) This latter modification, which uses a ferric ammonium sulfate color-developing reagent, enabled use of the ascorbate-preserved plasma samples. However, when this method was later compared with other methods, the estimates of plasma cholesterol are thought to be underestimated by 10–15%.

Other Measurements. Food intakes were obtained either from a questionnaire administered by a member of the provincial health survey team to all subjects who donated blood or from a 3-day weighing of actual food intakes in the households of urine and blood donors (27). Cadmium intake was estimated from an analysis (38) of county-based composites of foods of plant origin collected in each survey county [representative of both communes of the larger survey (27)]. These composites were comprised of proportions of foods actually consumed, as directly measured in the 3-day dietary survey. Further information concerning the questionnaire and the 3-day dietary survey is available in the paper by Chen et al. (27).

Statistical Methodology. Cumulative PLC mortality rates/1000 males aged 0–64 years for the 48 counties in which aflatoxin metabolites were analyzed were used as the outcome variable, both in the original geographic correlation study (39) and in the statistical analysis reported here. PLC mortality in persons <35 years of age was found to be marginal and no differences were found in the results using the 35–64 year age-truncated rates, thus confirming the validity of using rates for ages 0–64 years. This cumulative mortality rate is approximately equivalent to the cumulative risk of death by age 64 years from PLC in the absence of death from other causes. Age-standardized mortality rates for ages 35–64 years were also computed for meaningful comparison with the United States.

Pearson product-moment correlation coefficients were used to examine the relationship between PLC and selected variables including aflatoxin, HBsAg+, prevalence, and dietary intakes of salient foods. It should be emphasized that these zero-order correlation coefficients are cross-sectional measures of the degree of linear association between two factors and do not establish a causal relationship between these factors.

Multiple regression analysis was used to examine the combined effects of two or more factors on PLC and to determine the relationship...
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Table 1  
Age-truncated (35–64 years) primary liver cancer mortality rates in 48 rural Chinese counties and the United States

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>China (1973–1975)</td>
<td>82.5*</td>
<td>26.4</td>
</tr>
<tr>
<td>United States (1981–1985)</td>
<td>2.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* Average annual rate/100,000 population, age-standardized to world population (41).

between selected variables such as aflatoxin and PLC, adjusting for the effects of other relevant variables, such as HbsAg+ prevalence. Several such theoretically plausible model specifications were examined using this multivariate framework. Natural logarithmic transformations of PLC mortality rates were used in the analysis to obtain a normal distribution of the outcome variable for reliable statistical significance testing of the regression coefficients. For consistency, logarithms of the mortality rates were used in the computation of correlation coefficients as well. All probability levels of significance are two-sided.

RESULTS

The average age-truncated (35–64 years) PLC mortality rates in these 48 Chinese counties, when compared to the mean rate for the United States (40), with both rates being standardized to the world population (41), is 32-fold greater for males and 22-fold greater for females (Table 1). Mortality rates for males vary 39-fold from the highest county to the lowest county (Fig. 1), with the highest rates occurring along the eastern and southern coastal provinces (Fig. 2). Males have a higher prevalence than females, out_latitude females by 3.1-fold in China and 2.1-fold in the United States. A male partiality also has been observed in other societies (16, 42, 43), although the male to female ratio varies considerably (10).

Means and ranges for a few characteristics selected for their possible associations with PLC mortality rates for males are shown in Table 2. Also shown are comparable United States data (44–46) along with zero-order correlation coefficients showing associations of these characteristics with logarithmically transformed PLC mortality rates in China. Aflatoxin exposure exhibited wide variation when compared with previous studies. A much greater proportion of individuals were HBsAg+ carriers in China than in the United States. This very high prevalence of HBsAg+ in China (Fig. 3) is consistent with previous investigations showing carrier rates generally in excess of 10% for Sub-Saharan Africa and other areas of Southeast Asia (47, 48). The range of HbsAg+ prevalence in this study is rather more than previous studies. For example, Yeh et al. (26) observed a range of only 21.6–24.8% in 4 communes in the Guangxi Autonomous Region in southern China, while Peers et al. (19) reported a range of 21–28% in Swaziland. The prevalence of individuals positive for antibody to the HBV core protein (past infection) in these 48 counties was 18–81%, with a mean of 50% (data not shown). Plasma cholesterol levels were substantially below those for the United States; Chinese high values were near American low values. Geographic distribution of average (males and females) plasma cholesterol values for the 48 counties is shown in Fig. 4.

Intakes of all foods and about a dozen calculated nutrients were recorded, with corn, moldy peanuts, liquor, and cadmium selected for presentation in this paper. Information concerning other variables will be available in the report by Chen et al. (27). Liquor intake was considerably below that for the United States, with liquor contributing about two-thirds of total alcohol consumption (the remainder came from wine and beer). Consumption of moldy peanuts was expressed as the proportion of respondents having eaten this food during the past year. Data for moldy corn consumption also was sought, but this dietary practice was so uncommon that these data are not presented here. Instead, total corn intake was estimated from the quantity of ration dispensed by central government authorities. Corn as a cereal staple is consumed mostly in northern and central China, with mean daily intakes of 200 g or more for communes located in 10 of the 24 survey provinces; thus, substantial amounts are consumed by a large number of people in China. Estimated intakes of these foods were sought because they are considered the most common sources of mycotoxins, including aflatoxin.

Aflatoxin exposure was unrelated to PLC mortality (Table 2), as were the intakes of the traditional sources of aflatoxin contamination, corn and moldy peanuts. In contrast, prevalence of HBsAg carriers was highly significantly associated with PLC mortality (P < 0.001). The highly significant association of PLC mortality rates with total alcohol consumption was mostly attributed to liquor consumption. The intakes of several heavy metals (arsenic, cadmium, lead, mercury) were obtained from

Fig. 1. Cumulative liver cancer mortality rates for 0- to 64-year-old males in 48 survey counties.

Fig. 2. Geographic distribution of liver cancer mortality rates in 48 survey counties.

* M. Nui, Centers for Disease Control, Atlanta, GA, personal communication, 1989.
analyses of food samples collected in each county and only the intake for cadmium showed a significant association with PLC mortality rates. Mean cadmium intake was similar to an estimate of mean intake in the United States (46). Plasma cholesterol also was highly significantly associated with PLC mortality rates. As is well known, levels of this constituent may be related to dietary and other life-style conditions (49). In this study, even though it was very low compared to the United States, it tended to be associated with the intakes of foods of animal origin (Table 3). Significant correlations with plasma albumin and body height are consistent with diets richer in animal protein.

To determine whether the zero-order associations between PLC mortality and various characteristics (Table 2) might be accounted for by the simultaneous presence of other possible risk factors, regression analyses for selected model specifications on logarithmically transformed PLC mortality rates were carried out (Table 4). The simultaneous effects of all selected risk factors could not be validly assessed due to multicollinearity in the model. Thus, a series of models for which unbiased estimates could be obtained with the maximum number of independent variables were fitted to the data. Plasma cholesterol, HBsAg carrier prevalence, liquor intake, and cadmium intake were the most consistently associated PLC risk factors. Aflatoxin exposure consistently remained unassociated with PLC mortality regardless of model specification. The proportion of the variance in PLC mortality explained by the three most significant risk factors, HBsAg+ prevalence, plasma cholesterol, and cadmium intake, was 36% (model 8). Excluding aflatoxin did not alter this result, except in the biologically implausible opposite direction.

significantly associated with PLC mortality in all models. However, its contribution to the variation in PLC mortality was somewhat attenuated when plasma cholesterol and liquor intake were simultaneously added (models 4 and 5).

The most significant association with PLC mortality was with plasma cholesterol, regardless of model specification. Controlling for HBsAg+ prevalence, aflatoxin, liquor, cadmium, or any combination thereof still permitted a statistically significant plasma cholesterol association in each case. Daily liquor intake was associated significantly with PLC mortality when controlling for HBsAg+ prevalence and aflatoxin exposure ($\beta = 0.30$, $P = 0.073$), but this association disappeared when plasma cholesterol was included in the model (models 4 and 5). Cadmium intake also was associated with PLC mortality regardless of model specification. The proportion of the variance in PLC mortality explained by the three most significant risk factors, HBsAg+ prevalence, plasma cholesterol, and cadmium intake, was 36% (model 8). Excluding aflatoxin did not alter this result, except in the biologically implausible opposite direction.
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Table 3  Univariate correlation coefficients for various dietary and nutritional factors with average (males and females) plasma cholesterol in 65 rural Chinese counties

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary intake</td>
<td></td>
</tr>
<tr>
<td>Lipid (% kcal)</td>
<td>0.30*</td>
</tr>
<tr>
<td>Dietary fiber (g/day)</td>
<td>-0.27*</td>
</tr>
<tr>
<td>Dietary cellulose (g/day)</td>
<td>-0.37*</td>
</tr>
<tr>
<td>Total protein (g/day)</td>
<td>0.07</td>
</tr>
<tr>
<td>Animal protein (g/day)</td>
<td>0.24</td>
</tr>
<tr>
<td>Fish protein (g/day)</td>
<td>0.33b</td>
</tr>
<tr>
<td>Dairy (g/day)</td>
<td>0.21</td>
</tr>
<tr>
<td>Eggs (g/day)</td>
<td>0.21</td>
</tr>
<tr>
<td>Meat (g/day)</td>
<td>0.26b</td>
</tr>
<tr>
<td>Legumes (g/day)</td>
<td>-0.35b</td>
</tr>
<tr>
<td>Blood constituents</td>
<td></td>
</tr>
<tr>
<td>Plasma albumin (g/dl)</td>
<td>0.33b</td>
</tr>
<tr>
<td>Other characteristics</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.28b</td>
</tr>
</tbody>
</table>

a  P < 0.05.
b  P < 0.01.
* Data based on amount rationed in each county; mostly soy beans.

Table 4  Results of multiple regression analysis for various model specifications on male primary liver cancer mortality

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables</th>
<th>β</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aflatoxin</td>
<td>-0.22</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>HBsAg+</td>
<td>0.32</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>0.37</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>(R² = 0.34, P = 0.0007)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Aflatoxin</td>
<td>-0.11</td>
<td>0.405</td>
</tr>
<tr>
<td></td>
<td>HBsAg+</td>
<td>0.28</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>Liquor</td>
<td>0.30</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>(R² = 0.28, P = 0.0036)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Aflatoxin</td>
<td>-0.18</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td>HBsAg+</td>
<td>0.28</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>0.31</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>(R² = 0.30, P = 0.0015)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>HBsAg+</td>
<td>0.20</td>
<td>0.188</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>0.27</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>Liquor</td>
<td>0.16</td>
<td>0.338</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>0.23</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td>(R² = 0.37, P = 0.0006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Aflatoxin</td>
<td>-0.18</td>
<td>0.185</td>
</tr>
<tr>
<td></td>
<td>HBsAg+</td>
<td>0.25</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>0.31</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>Liquor</td>
<td>0.15</td>
<td>0.375</td>
</tr>
<tr>
<td></td>
<td>(R² = 0.35, P = 0.0014)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Aflatoxin</td>
<td>-0.22</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>HBsAg+</td>
<td>0.25</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>0.34</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>0.27</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>(R² = 0.41, P = 0.0002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Aflatoxin</td>
<td>-0.13</td>
<td>0.330</td>
</tr>
<tr>
<td></td>
<td>HBsAg+</td>
<td>0.25</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td>Liquor</td>
<td>0.22</td>
<td>0.170</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>0.25</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>(R² = 0.33, P = 0.0018)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>HBsAg+</td>
<td>-0.26</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol</td>
<td>0.31</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>0.27</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>(R² = 0.36, P = 0.0003)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Logarithm of cumulative PLC mortality rates for males aged 0–64 years.

DISCUSSION

A comparison of the experimental features of this survey with the most convincing cross-sectional surveys previously reported is shown in Table 5. The first four and, more recently, the first five studies (22, 50–53) listed in Table 5 have been widely cited (5, 7, 9, 10, 23, 52–56) and the data from these studies often have been combined (7, 52–56) to show an impressive correlation of PLC mortality rates with aflatoxin intake; the experimental features of the combined data also are shown. Within these individual studies, all considered by the 1987 IARC report (5), the range of aflatoxin intake was 3- to 11-fold, the range of mortality rates was 3- to 4-fold, and the number of survey sites was 2–8. However, in none of these studies was HBsAg+ prevalence simultaneously surveyed, although a later study in Kenya (57) found a relatively constant HBsAg+ prevalence for the three survey regions in the study by Peers and Linsell (51). The recent study in Swaziland (19), also considered by the 1987 IARC report, was the only one that recorded both aflatoxin exposure and HBsAg+ prevalence; no independent HBsAg+ effect was observed. Also shown for comparison are the more recent studies of Yeh et al. (26) and Autrup et al. (12), each of whom simultaneously measured aflatoxin exposure and HBsAg+ carrier rates.

The experimental features of our China survey sharply contrast with the experimental features of these various cross-sectional studies. There is a much larger number of survey sites, a wider range of PLC mortality rates, a wider range of aflatoxin exposure, simultaneous measurement of a generous range of HBsAg+ prevalence, and a large array of possible risk factors (more than 250 nutritional, dietary, and lifestyle variables). An improved method of estimating aflatoxin exposure also was used. Measurement of aflatoxin metabolites in urine directly measures exposure and integrates intake over a day or so, while measurement of intake by aflatoxin analysis of foods is subject to the highly erratic distribution of aflatoxin in different foods (58).

The lack of an association between aflatoxin exposure and PLC mortality in this China study is particularly remarkable in view of the findings of most previous investigations. The absence of an aflatoxin-PLC association is consistent with a similar lack of association of PLC mortality with the consumption of the two foods most commonly contaminated with aflatoxin, corn and moldy peanuts; this consistency is particularly notable because of the uniqueness of each method for estimating aflatoxin exposure. A power calculation, undertaken in retrospect, shows that, with 48 counties and an α level of 0.05, a correlation coefficient of +0.48 is required for significance; the observed coefficient of −0.17 is far below this reference point. This nonassociation between aflatoxin intake and PLC mortality also persists after controlling for potential confounding factors such as plasma cholesterol, prevalence of HBsAg+, and intakes of alcohol and cadmium.

In contrast to the lack of an association with aflatoxin, PLC mortality was highly correlated with HBsAg+ prevalence. PLC mortality was not correlated with past HBV infection (r = −0.08), as assessed by the prevalence of antibody to the HBV core protein, (data not shown). These relationships between PLC mortality and persistent and past HBV infection are consistent with the hypotheses and data of Blumberg and Lon (58).

The association of PLC mortality rates with liquor intake, making up about 80% of total alcohol intake, is in accord with similar observations of total alcohol intake (25, 59, 60). However, the liquor association was eliminated when controlling for plasma cholesterol, as in models 4 and 5 of Table 4. If some of the liquor effect was explained by higher plasma cholesterol, then this implies that a sustained intake of liquor may be associated with elevated plasma cholesterol. Indeed, such a relationship has been reported (61).

The association of PLC mortality with plasma cholesterol is especially interesting. Inspection of the partial regression coef-
Table 5 Comparison of strengths of principal ecological studies to detect contributions of aflatoxin intake and HBsAg+ prevalence to PLC risk

<table>
<thead>
<tr>
<th>Report</th>
<th>No. of survey districts</th>
<th>Country</th>
<th>Range of PLC mortality rate</th>
<th>Range of aflatoxin intake</th>
<th>Range of HBsAg+ prevalence</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-IARC (1987)</td>
<td>2</td>
<td>Thailand</td>
<td>3.0</td>
<td>9.0</td>
<td>*</td>
<td>Shank et al., 1972 (50)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Kenya</td>
<td>4.2</td>
<td>3.0</td>
<td></td>
<td>Peers and Linsell, 1973 (51)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Mozambique</td>
<td>4.2</td>
<td>8.5</td>
<td></td>
<td>Peers et al., 1976 (22)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Swaziland</td>
<td>3.5</td>
<td>11.1</td>
<td></td>
<td>Van Rensburg et al., 1985 (53)</td>
</tr>
<tr>
<td>10</td>
<td>Combined data (first 4 studies)</td>
<td>10.8</td>
<td>25.6*</td>
<td></td>
<td></td>
<td>Shank et al., 1972 (50)</td>
</tr>
<tr>
<td></td>
<td>Combined data (first 5 studies)</td>
<td>14.8</td>
<td>52.4</td>
<td></td>
<td></td>
<td>Peers and Linsell, 1973 (51)</td>
</tr>
<tr>
<td>Post-IARC (1987)</td>
<td>10*</td>
<td>Swaziland</td>
<td>8.6</td>
<td>13.9*</td>
<td>1.3</td>
<td>Peers et al., 1987 (19)</td>
</tr>
<tr>
<td></td>
<td>8*</td>
<td>Kenya</td>
<td>11.2 (M)*</td>
<td>3.5 (M)*</td>
<td>4.7 (M)</td>
<td>Autrup et al., 1987 (12)</td>
</tr>
<tr>
<td></td>
<td>5*</td>
<td>Guangxi, China</td>
<td>3.5</td>
<td>172.0</td>
<td>1.07</td>
<td>Yeh et al., 1989 (26)</td>
</tr>
<tr>
<td>48</td>
<td>China</td>
<td>39.0</td>
<td>600+*</td>
<td>28.0</td>
<td></td>
<td>Chen et al., 1990 (27)</td>
</tr>
</tbody>
</table>

* Range based on low value of 3.5 mg/kg body weight/day from Shank et al. (50) and high value of 89.5 mg/kg body weight/day in 1985 paper of Van Rensburg et al. (53).

The link between plasma cholesterol and diets containing more foods of animal origin was somewhat surprising in view of the low plasma cholesterol and the low intake of such foods in China. Plasma cholesterol in this cohort ranged up to about 190 m/kg/dl (for pools of individuals and after correction for methodological underestimate) which is near the low end of the range for comparable Western subjects. And mean intake of animal products in China was quite low; for example, these foods contributed, on average, only about 11% of the total protein intake in the 65 counties of the parent survey (27).

The finding that elevated plasma cholesterol and increased prevalence of HBsAg+ were significant risk factors for PLC mortality, while aflatoxin was not a risk factor, is consistent with data from animal studies. When groups of animals all fed the same level of dietary protein (20% casein) were given increasing doses of aflatoxin, the expected dose-response relationship was observed for the formation of presumptive preneoplastic liver lesions (39). In contrast, when animals were fed either lower levels of 5–10% animal protein (39, 65, 66) or the same level (20%) of plant protein (67) after completion of aflatoxin dosing, development of preneoplastic lesions and tumors was markedly inhibited. Both the promotion and progression stages of aflatoxin-induced tumor development may be readily modulated by the level and type of dietary protein intake, completely eliminating the dose-dependent response reduced by the level of aflatoxin intake (68). In these low protein fed animals, plasma cholesterol also was reduced. The level of dietary protein required for inhibition of hepatic foci and hepatic tumor development (<10% protein) is near the level required for maximum growth rate (69) or, said another way, is the amount generally available in a diet mostly comprised of foods of plant origin.

The mean level of protein intake in the Chinese cohort, at 0.99 g/kg body weight, is modestly above the United States recommended dietary allowance of 0.8 g/kg (70) and significantly below the mean of 1.18 g/kg for comparable American adult males (71). The more significant difference in protein intake between these populations, however, is the ratio of plant to animal protein (including fish). In China animal protein represents only about 11% of the total (8% nonfish and 3% fish), while in the United States it is about 70% of the total or about 0.13 and 0.82 g/kg body weight/day, respectively [see Chen et al. (27) for estimation details]. The intake of efficiently utilized protein required for good growth both in rats and in people, when expressed per unit body weight, are very similar: about 9–10% of total dietary protein for both species, an amount approximately equivalent to the recommended dietary allowance (70). Even though the mean intake in China is close

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to this recommended intake, about one-half of the population consume more. If this extra dietary protein were to be plant protein consumed as part of a simple diet (that is, a diet with little food variety), its adverse effects on PLC mortality likely would be limited; however, if the extra protein were to be obtained even from small additions of animal protein, measurable risk for PLC mortality may occur.

The finding of a nonassociation between aflatoxin intake and PLC mortality, which contrasts with the results of many previous studies, is provocative. An explanation of this difference may be considered from several points of view. First, Chinese might respond differently to aflatoxin, perhaps because of unique genetic or environmental characteristics. While this explanation never can be fully discarded, very likely it is quite untenable, first, because some of the previous data interpreted as showing a positive association between aflatoxin intake and PLC mortality were based on investigations of Chinese subjects (18, 26) and, second, because major ethnic differences in risk for other cancers are greatly reduced or eliminated after migration to new environments.

A second argument could be that the null effect in this study may have occurred because measurement of aflatoxin exposure during the survey period was not representative of past intakes when the cancers were forming. Although this possibility cannot be discounted, it should be remembered that a similar limitation also existed for all previous studies used to justify aflatoxin as a human carcinogen, especially those which relied on aflatoxin analysis of food samples and use of food disappearance records. In fact, this study in China, aflatoxin exposure very likely is more reliable because of the analysis of urinary aflatoxin metabolite excretion which directly represents and integrates over a day or so actual consumption. Moreover, aflatoxin contamination rates in a county in the Guangxi Autonomous Region were relatively stable during the years 1972–1983 (see above). In a study documenting long-term aflatoxin exposure in the United States, no association with PLC was observed (23).

A third interpretation of these data suggests that aflatoxin may not be a significant human carcinogen. Three types of evidence support this view. First, the statistical power and more comprehensive range, diversity, and inclusiveness of risk factors available in this study far surpasses not only similar features of previous individual cross-sectional studies but also substantially surpasses their combined experimental features (Table 5). Second, the data from this study in China, which corresponds with laboratory animal observations, may be explained by a biologically plausible hypothesis. And third, these data suggest human resistance to this animal carcinogen, a finding which is supported by in vitro aflatoxin studies on species of varying resistance (72–74). Also, humans may be further refractory when consuming lower protein diets. [Incidentally, inhibition of aflatoxin-induced liver cancer with consumption of low protein diets should not be confused with an enhancement of aflatoxin-induced acute toxicity (75). Under these conditions of low protein feeding, it has been hypothesized that there is an accumulation of unmetabolized parent compound which inhibits cell respiration to cause cell death (76), a hypothesis which is also supported by the reports of acute toxicity in protein-malnourished children fed aflatoxin-contaminated foods (77).]

Although it might be argued that the China data are more convincing because of the supporting experimental animal and biochemical evidence concerning the effects of nutrition and natural species resistance and because of the considerable advantage in experimental parameters, such a conclusion is not fully satisfying. Even if the previous studies were much more limited in experimental design features, positive aflatoxin-PLC associations nonetheless were reported and must be explained.

In an attempt to provide an explanation for these differing results, we offer the following review of interpretations given to previous investigations and reviews, particularly the conclusions drawn by the 1987 IARC report (5). Unfortunately, several misinterpretations and misstatements of fact were found in this 1987 IARC report (5) and some of these were noted earlier. These shortcomings, alone, cast doubt on the revised conclusion that there was sufficient evidence to conclude aflatoxin human carcinogenicity.

The most compelling evidence in favor of aflatoxin human carcinogenicity is the high correlation obtained [r = 0.88, (53)] for aflatoxin intake and PLC mortality when the results from the first five studies of Table 5 are combined. Such a correlation is impressive, especially when considering the rather severe experimental limitations of the individual studies. One of the more severe limitations noted by others (10, 78) is the absence of data concerning HBV infectivity; none of these studies obtained this information, although one (51) provided complimentary data only in retrospect (57). If an independent aflatoxin effect is hypothesized, as suggested by the data, then it is necessary to assume, among other assumptions, that HBsAg+ prevalence is relatively constant among the 17 survey sites of these studies (that is, if this virus is to be considered an important risk factor). This assumption seems unlikely. For example, later studies in these same regions showed that HBsAg+ carrier prevalence rates were quite variable, being 5–23% in Kenya (12) and 5–35% in Swaziland (19) which are similar to the 1–28% range observed by us in China (27). Variation of this magnitude offers the very real possibility that the observed aflatoxin effect may be attributed to HBsAg+ carrier prevalence, especially when this variation was reported for the same survey regions which supplied data points for the combined regression. Furthermore, our calculation indeed shows that, in the study of Peers et al. (19), aflatoxin intake and HBsAg+ prevalence are significantly correlated (r = 0.73, P < 0.05). The study of Yeh et al. (26) of four sites in Guangxi, China, is the only report showing an aflatoxin effect on PLC risk in the presence of a reasonably constant HBsAg+ carrier prevalence (21.6–24.8%). However, these prevalence data contrast with those of Chen et al. (27) who included four counties in the same Guangxi Autonomous Region and found variable prevalence rates of 8–23% (50 subjects/county, 50% of each sex) (indeed, one county, Fusi, was included in both studies, with a 23% prevalence of HBsAg+ in both investigations). In summary, considerable variation in HBsAg+ prevalence rates are likely to occur both in Africa and southeast China, and furthermore, these rates may correlate with aflatoxin intake (19), thus confounding observed relationships between aflatoxin and PLC.

In the earlier studies published prior to the 1987 IARC report (5), numerous other shortcomings (crude estimates of aflatoxin intake, disease diagnosis errors, incomparable population bases, etc.) have been noted by other commentators (10, 78) but errors of this type should not negate the aflatoxin-PLC correlations observed; such shortcomings would only add "noise" to the analysis. Another explanation of the observed aflatoxin effect is the possibility that aflatoxin exposure is a surrogate measure for the presence of other unmeasured risk factors, including, for example, alcohol, cadmium, and plasma choles-
tropol or its dietary determinants, as illustrated in the China data base. Surrogate representation, however, would require relatively constant exposure to these unknown factors among these various studies.

An analysis of these data reported here would not be complete without careful comparison with three recent studies (12, 19, 26) each of which was cross-sectional in design and each of which included simultaneous measurement of PLC mortality, HBsAg+ carrier rates, and aflatoxin exposure. One study (19) was cited by the 1987 IARC report (5) and one (26) is being considered by current committees (United States Food and Drug Administration, California Proposition 65 Risk Assessment Committee, and industry groups) as a very important adjunct to the 1987 IARC report (5). Aflatoxin exposure was associated with PLC mortality in the studies of Yeh et al. (26) and Peers et al. (19) but not in the study of Autrup et al. (12) (except a smaller indeterminant subset of the population). Comparisons of a few statistical parameters for these studies are shown in Table 6. HBsAg+ carrier rates for males were comparable for Yeh et al. (19.5–24.8%) and Peers et al. (20–35%), both being somewhat higher than Autrup et al. (5%-23%). The studies of Yeh et al. (26) and Peers et al. (19) afford the most appropriate comparison, both because of comparable mean HBsAg+ prevalence and because both studies observed a highly significant aflatoxin effect. However, in other ways, the results of these two studies were inconsistent as illustrated by comparison of the respective regression equations (Table 6). Although the slope coefficients (0.15 and 0.12) were reasonably similar, the intercepts (mortality rates in absence of aflatoxin exposure) were markedly different. The most likely explanation might be that the mean HBsAg+ carrier rates were much higher in the study by Yeh et al. (26), but this does not appear to be true; if so, then this discrepancy might be that the mean HBsAg+ carrier rates were much higher in the study by Autrup et al. (26) than in the study by Peers et al. (19). Thus, there appears to be a major discrepancy in PLC rates between southern China and east Africa (i.e., different intercepts) that cannot be attributed either to a difference in the observed HBsAg+ prevalence or to a difference in aflatoxin exposure. Another discrepancy apparent from a comparison of these studies is the findings of Peers et al. (19) who observed a significant HBsAg+ effect on PLC mortality; in contrast, Yeh et al. (26) saw no HBsAg+ effect in the cross-sectional analysis, although they did observe a highly significant effect when comparing cases and controls within their cohort. And finally, the combined data of the previous cross-sectional studies (pre-1987 IARC report) exhibit a somewhat similar intercept to that of Peers et al. (19); in fact, these combined data (17 points) include 4 survey sites representing the 10 subregions included in the later Peers et al. (19) study. However, the slope constant of the combined studies is nearly triple the slope constant for the study by Peers et al. (19).

The third recent study was that by Autrup et al. (12) who found no aflatoxin effect on PLC incidence rates when all ethnic, social, and cultural groups were included in the analysis (our calculation of the correlation coefficients for males and females actually showed inverse associations, —0.40 and —0.35, respectively), but a significant positive association was noted when the analysis was limited to the Bantu people (no data were given on the apparently small number of these people). Not only was there no aflatoxin effect in this study (12) but also there was no HBsAg+ effect.

When all previous studies, before and after the 1987 IARC report (5), are considered, there is substantial uncertainty concerning the role of aflatoxin in the etiology of human PLC. At a minimum, we believe that it is definitely not possible to conclude that there is sufficient evidence for human carcinogenicity (5), particularly because the criteria of “chance, bias and confounding” cannot “be ruled out with reasonable confidence” as required by the IARC guidelines (5). Also adding doubt on the sufficiency of evidence are the observations, first, that the human species is highly resistant when compared in vitro to other species (72-74) and, second, that the dose-response relationship between aflatoxin exposure and PLC rates reported for humans is asymptotic to an upper PLC limit (concave downward) which is opposite to that observed for the relationship between aflatoxin and putatively preneoplastic liver lesions in experimental animals (concave upward) (68).

Comparison of these previous studies illustrates serious difficulties of interpretation which are not easily resolved and no new information concerning causality is apparent. In contrast, the study by Chen et al. (27) indicates highly significant risk

**Table 6 Statistical parameters* and results of three comparable studies on aflatoxin exposure, HBsAg+ carrier rate and PLC mortality**

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of sites</th>
<th>AF vs. PLC</th>
<th>HBsAg+ vs. PLC</th>
<th>AF vs. HBsAg+</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peers et al. (1987)</td>
<td>10</td>
<td>0.84*</td>
<td>0.73*</td>
<td>0.73*</td>
<td>PLC = 4.1 + 0.12 AF</td>
</tr>
<tr>
<td>Autrup et al. (1987)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>8</td>
<td>—0.40(NS)</td>
<td>0.17(NS)</td>
<td>0.23(NS)</td>
<td>PLC = 3.7 — 0.05 AF</td>
</tr>
<tr>
<td>Yeh et al. (1989)</td>
<td>5</td>
<td>0.996*</td>
<td>0.28(NS)</td>
<td>0.00(NS)</td>
<td>PLC = 159 + 0.15 AF</td>
</tr>
<tr>
<td>Combined data</td>
<td>17</td>
<td>0.85*</td>
<td></td>
<td></td>
<td>PLC = 8.7 + 0.32 AF</td>
</tr>
</tbody>
</table>

* All parameters calculated from data in original papers.

* AF, aflatoxin; NS, not significant (P > 0.05).

* Original 5-year PLC incidence rates for males, annulled and truncated for ages 15–64 years. Aflatoxin exposure measured as µg/person/day converted to ng/kg body weight/day assuming 70 kg body weight.

* P < 0.01.

* P < 0.05.

* Original PLC incidence rates converted to truncated rates for ages 15+ years; rates in Table 5 of Autrup et al. (12) divided by 0.61 to account for 39% of total population <15 years (based on demographic distribution for Senegal as a representative rural country (41) and assuming no PLC cases <15 years). Aflatoxin-guanine adduct in urine assumed to be 1% of aflatoxin intake by Autrup et al. (12).

* PLC incidence rates reported for males aged 25–64 years. Aflatoxin exposure of mg/person/day converted to ng/kg body weight/day assuming 49 kg body weight [recorded in same area by Chen et al. (27)]. Aflatoxin data not available for one site.

* No HBsAg+ data available. Originally published mortality rates made equivalent as follows: Shank et al. (50), original rates (all ages, both sexes) converted to truncated rates for 15+ years, assuming 4:1 male:female PLC incidence ratio, 1:1 sex distribution; and age distribution of Senegal (41) as representative of a rural developing country; Peers et al. (22), original rates (all ages, males only, ages 15–64 years) used directly from Table 5; Peers et al. (51), original rates (all ages, males only, ages 15+ years), used directly from Table 4; Van Rensburg et al. (53), original rates (all ages, males only) converted to truncated rates, 15+ years, using Senegal (41) age distribution.

*P < 0.001.
exposure will be estimated by measurement of circulating PLC risk. This study also offers the advantage that aflatoxin intake over a period of time even longer than that used in the China study reported here. These results are eagerly awaited.

In view of the comprehensive and reliable nature of the China data, we propose the following model to explain the etiology of PLC. For a given population, the vast majority of individuals who are susceptible to PLC are mostly limited to those who remain persistently infected with HBV, a conclusion drawn from the extraordinary PLC risk observed for HBsAg+ carriers (16). Thus, the first approximation of population risk of PLC is the prevalence of HBsAg+ carriers. Then, within the HBsAg+ carrier population, additional risk is contributed chiefly by nutritional and dietary practices that enhance liver cell proliferation, such as with diets containing significant amounts of animal protein. In contrast, aflatoxin contributes little or no PLC risk. Even though aflatoxin may act as a carcinogetic initiator, undoubtedly it contributes only a very small proportion of the initiating activity routinely exposing the liver. Innumerable dietary constituents, upon metabolic activation (mostly in the liver itself), may be genotoxic (80). Even modestly small amounts of these substances should be able to cause enough initiation for tumor occurrence provided that, subsequently, there is adequate nutrient activity to cause liver cell proliferation. The HBV virion also may be capable of causing initiation through insertion of its DNA into liver cell DNA (81).

This model suggests that HBsAg+ positivity is a necessary but insufficient cause of PLC, that aflatoxin is an unnecessary and insufficient cause, and that sustained nourishment causing liver cell proliferation (and elevated plasma cholesterol) is a necessary and insufficient cause for HBsAg negative carriers but a necessary and sufficient cause for HBsAg+ carriers. Thus, PLC may occur among HBsAg+ carriers consuming no aflatoxin but sufficient amounts of a nutrient-dense diet that stimulates liver cell proliferation. There is also the possibility that such a diet first could stimulate replication of the inoculating virus which then causes liver cell proliferation.6

An obvious question concerning the effect of "enriched" nutrition upon PLC etiology is: why is this disease so much more common in undernourished and impoverished societies? According to this model, PLC is more common because HBsAg+ carriers are more common, not because undernourishment is more common. Then, among the HBsAg+ carrier population, there is additional risk for those who consume significant amounts of dietary constituents, such as animal protein, that encourage liver cell proliferation. Even though, within these societies, a large number of people consume nutritionally poor diets containing minimal food variety and little or no animal protein, enough of the HBsAg+ carriers still consume enough animal protein (or total mixed protein and companion nutrients) to elevate plasma cholesterol, induce liver cell proliferation, and promote tumor development. HBsAg+ carriers are likely to be particularly vulnerable to PLC even with modest nutritional enrichment. For aflatoxin to contribute significant risk, consumption must be high enough and prolonged enough to cause significant liver cell proliferation.

These hypothesized relationships suggest that, in addition to the HBsAg+ carrier rate, the level of animal protein intake or similar nourishment would be the next most important determinant of PLC risk. This would be indicated by increasing prevalence of PLC among HBsAg+ carriers who consume increasing amounts of animal protein. Some evidence for this relationship is indicated by a crude comparison of PLC prevalence among HBsAg+ carriers in Swaziland [estimated 1.8% prevalence with negligible intake of animal protein, as indicated in report of Peers et al. (19)], China [16% prevalence with low but significant animal protein intake (27)], and Taiwan [40-50% prevalence (16) with medium animal protein intake (82)].

This model emphasizes (a) the role of liver cell proliferation in tumor development, recently emphasized by others (83, 84), (b) the abundance of dietary constituents capable of causing liver cell initiation (80), (c) the great risk for PLC among HBsAg+ carriers (14), and (d) the ability of animal protein (perhaps also other nutrients) to modulate tumor occurrence (even after relatively modest initiation may have occurred).

This model is not unlike that observed for alcoholics who incur a higher risk for liver cancer after withdrawal of alcohol than before. Lee (85), for example, concluded from his results that after "liver disease is established (as with alcohol), and with the substitution of a nutritious diet and a reduced alcohol intake, circumstances may be more favourable for the development of hepatoma." Accordingly, with HBV infection, if immunity to HBV infection cannot be established early to eliminate persistent infection, then care must be used thereafter to minimize the consumption of a nutritionally rich (animal protein, fat) diet.

And finally, this explanation is in harmony, nutritionally speaking, with the preventive effect of nutrition upon the development of other cancers (86).

**ACKNOWLEDGMENTS**

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* S. Broder, personal suggestion.
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Nonassociation of Aflatoxin with Primary Liver Cancer in a Cross-Sectional Ecological Survey in the People's Republic of China

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