Letters to the Editor

Correspondence re: G-S. Qian, *et al.*, A Follow-Up Study of Urinary Markers of Aflatoxin Exposure and Liver Cancer Risk in Shanghai, People's Republic of China. Cancer Epidemiol., Biomarkers & Prev., *3:* 3–10, 1994, and C. C. Harris, Solving the Viral-Chemical Puzzle of Human Liver Carcinogenesis. Cancer Epidemiol., Biomarkers & Prev., *3:* 1–2, 1994

Letter

T. Colin Campbell

Division of Nutritional Biochemistry, Cornell University, Ithaca, New York 14853

Qian *et al.* (1) showed an impressive relative risk of 59.4 for HCC[1] individuals who were both positive for HBsAg and who excreted in their urine elevated levels of aflatoxin metabolites when compared with individuals who were positive for HbsAg but negative for aflatoxin exposure (relative risk of 7.3, unusually low for HBsAg carriers). Thus, they concluded that "these data indicate that aflatoxin exposure may be an important risk factor for liver cancer in this high-risk population" and go on to say that "studies such as this one demonstrate the extraordinary potential of molecular biomarkers for individual risk quantification."

While these findings may indicate important new evidence for the hepatocarcinogenicity of aflatoxin for humans they also may not. For example, the HCC risk putatively attributed to aflatoxin appears to be accounted for mostly by the AFB-N^7-gua adduct (see their Table 1), thus leading the authors to conclude not only that AFB_1 is a risk factor but also that urinary AFB-N^7-gua may be the most reliable biomarker of the urinary aflatoxin metabolites measured. However, I question the reliability of this conclusion. The portion of the HCC risk attributed to AFB_1 and primarily accounted for by AFB-N^7-gua could also be caused by factors which enhance enzymatic activation of AFB_1 by the hepatic P-450 enzyme system to produce more AFB-N^7-gua, subsequently to be excreted in the urine. These enzyme-inducing factors could readily be nutritional (2), especially those which also are associated with elevated plasma cholesterol.

This interpretation is in accord with our finding for 48 survey counties in an ecological study in China (3) that the most significant and robust determinants of HCC risk were elevated cholesterol levels and HbsAg positivity, not aflatoxin exposure, a finding made plausible by essentially identical data obtained from our experimental animal studies (4). This interpretation is also in accord with our experimental animal observations, made nearly 20 years ago (5), that *in vivo* activation of AFB_1 to form hepatic DNA adducts could be markedly enhanced (3–4-fold) by a modest elevation in the intake of animal protein, along with a corollary elevation of plasma cholesterol (not to be confused with the inverse relationship between plasma cholesterol and HCC (6) for individuals whose cholesterol concentrations probably were lower due to incipient, clinically undetected disease).

This nutrition-focused hypothesis, if correct, would yield two very different conclusions, both of which are essentially related to the same nutritional cause: (*a*) AFB-N^7-gua would be only a surrogate biomarker for a nutritional effect on AFB_1 activation, not a biomarker for the risk-enhancing effect of AFB_1; and (*b*) the extraordinarily high relative risk observed for the combined exposures to HBsAg antigenicity and AFB_1 could also be due to this same nutritional effect. For example, we have recently shown that the same modest animal protein intake which markedly elevates AFB_1 activation also markedly increases the overexpression of a hepatitis B virus transcript in mice (7). Thus, a cause (in this case, animal protein and its multiple dietary correlates) which simultaneously underlies both activities, AFB_1 activation and hepatitis B virus-DNA overexpression, could readily promote a higher order combined relative risk. Accordingly, AFB_1 exposure for all individuals, cases, and controls would be relatively random (certainly highly variable) and could be a relatively insignificant HCC risk factor, especially for humans, for whom *in vitro* metabolism studies indicate unusual species resistance (see Ref. 8 and earlier papers).

Until this hypothesis of a nutritional effect is ruled out (admittedly, it is not yet fully consistent with all data), I would suggest that the authors temper their enthusiasm for the "extraordinary potential of molecular biomarkers" as a tool for the determination of aflatoxin-attributable HCC risk for individuals. Considering all the evidence, I still favor the hypothesis that HCC is primarily a viral-nutritional disease, not a viral-chemical carcinogen disease. Accordingly, the editorial by Harris (9) in this same issue is woefully remiss in not taking into consideration the unusually robust effect of nutrition on this and other cancers. Meager and uncritical mention of selenium deficiency as a possible nutritional risk factor treats the subject of nutritional carcinogenesis unusually superficially.

Received 2/9/94; accepted 6/15/94.

[1] The abbreviations used are: HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; AFB-N^7-gua, aflatoxin N^7-guanine; AFB_1, aflatoxin B_1.

References

1. Qian, G-S., Ross, R. K., Yu, M. C., Yuan, J-M., Gao, Y-T., Henderson, B. E., Wogan, G. N., and Groopman, J. D. A follow-up study of urinary

markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol., Biomarkers & Prev., *3:* 3–10, 1994.

2. Campbell, T. C., and Hayes, J. R. Role of nutrition in the drug metabolizing system. Pharmacol. Rev., *26:* 171–197, 1974.

3. Campbell, T. C., Chen, J., Liu, C., Li, J., and Parpia, B. Nonassociation of aflatoxin with primary liver cancer in a cross-sectional ecological survey in the People's Republic of China. Cancer Res., *50:* 6882–6893, 1990.

4. Youngman, L. D., and Campbell, T. C. Inhibition of aflatoxin B1-induced γ-glutamyl transpeptidase positive (GGT+) hepatic preneoplastic foci and tumors by low protein diets: evidence that altered GGT+ foci indicate neoplastic potential. Carcinogenesis (Lond.), *13:* 1607–1613, 1992.

5. Preston, R. S., Hayes, J. R., and Campbell, T. C. The effect of protein deficiency on the *in vivo* binding of aflatoxin B_1 to rat liver macromolecules. Life Sci., *19:* 1191–1198, 1976.

6. Chen, Z., Keech, A., Collins, R., Slavin, B., Chen, J., Campbell, T. C., and Peto, R. Prolonged infection with hepatitis B virus: a factor contribution to the association between low blood cholesterol and liver cancer. Br. Med. J., *306:* 890–894, 1993.

7. Hu, J., Chisari, F. V., and Campbell, T. C. Modulating effect of dietary protein on transgene expression in hepatitis B virus (HBV) transgenic mice. Proc. Am. Assoc. Cancer Res., *35:* 104, 1994.

8. Booth, S. C., Bosenberg, H., Garner, R. C., Herzog, P. J., and Norpoth, K. The activation of aflatoxin B1 in liver slices and in bacterial mutagenicity assays using livers from different species including man. Carcinogenesis (Lond.), *2:* 1063–1068, 1981.

9. Harris, C. C. Solving the viral-chemical puzzle of human liver carcinogenesis. Cancer Epidemiol., Biomarkers & Prev., *3:* 1–2, 1994.

Reply

John Groopman, Curtis Harris, Brian Henderson, Ronald Ross, Gerald Wogan, and Mimi Yu

Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, Maryland 21205 [J. G.]; Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20892 [C. H.]; Salk Institute for Biological Studies, La Jolla, California 92037 [B. H.]; University of Southern California, Los Angeles, California 90033 [R. R., M. Y.]; and Division of Toxicology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 [G. W.]

For the past two decades there has been intense research about the causes of human liver cancer, a disease that results in at least 200,000 deaths each year. Liver cancer is a major public health problem in sub-Saharan Africa and China. In some counties in China this disease is responsible for 10% of all deaths each year. Thus, there is an acute need for the design of preventive interventions to lessen the incidence of this disease in these populations. Clearly, this will be hastened by the identification of the major etiological factors in liver cancer and the subsequent development of primary or secondary prevention strategies to lower exposure to these risk factors.

There is an overwhelming amount of experimental data across species and in experimental models demonstrating the potency of aflatoxin B_1 as a carcinogen and mutagen (reviewed in Ref. 1). Further, there is definitive evidence that people have the metabolic capacity to activate aflatoxin to the same DNA-damaging products that are causally related to cancer in experimental models. Thus, there are no mechanistic reasons to believe that aflatoxins are inert substances for people.

A well established major risk factor for liver cancer is hepatitis B virus. Unfortunately, there are no animal models and very few cell culture systems with which to explore the mechanistic basis for the tumorigenicity of the human virus. Indeed, the causal association between HBV[1] and human liver cancer was established entirely by descriptive, case-control and, especially, prospective epidemiological investigations. These studies were only possible with the development of biomarkers for HBV-specific antigen and antibody levels in human samples. The strong association between the hepatitis B biomarker levels and human liver cancer has been shown many times. However, there is at least a 5–8-fold variation in liver cancer incidence across regions of the world where the prevalence of hepatitis B viral markers is comparable. Thus, it is reasonable to expect that liver cancer, like all human diseases, will have a multifactorial etiology.

The recent development of molecular biomarkers for aflatoxins has permitted the investigation of the independent role of aflatoxins in human liver cancer development as well as the potential interaction between hepatitis B virus and dietary aflatoxins. In the original study reported by Ross *et al.* (2) and followed up in Qian *et al.* (3), there was for the first time the use of aflatoxin-specific biomarkers and HBV markers in the same investigation. In addition to providing the first direct evidence in human studies that aflatoxins are major risk factors for hepatocellular carcinoma, another conclusion of these studies was the existence of a synergistic interaction between HBV and aflatoxin exposure, as assessed by urinary biomarkers. Further, there is evidence that some of the urinary aflatoxin biomarkers may be stronger predictors of risk than others. These findings await confirmation from further follow-up investigations in this and other populations.

In his letter, Dr. Campbell raises several issues about the role of serum cholesterol in liver cancer pathogenesis and the possibility that dietary protein can alter risk by affecting levels of the cytochrome P-450s that activate aflatoxin B_1 to produce DNA adducts. There are no data in human studies, to our knowledge, that provide any direct support for these hypotheses. His findings in an ecological study in China on the relation of serum cholesterol to liver cancer lack a credible biological mechanism but unlike our prospective study in Shanghai, correlational studies are well known to be highly limited in their ability to address cause and effect relationships. In fact, the lack of correlation in his studies between aflatoxin exposure and liver cancer may be completely related to the ecological study design. The nested case-control studies reported in Ross *et al.* (2) and Qian *et al.* (3) are prospective in nature and directly link exposure to outcome in specific individuals.

As part of our cohort study in Shanghai, a questionnaire with detailed questions on current dietary habits was administered to each study subject. Thus, we were able to directly test Campbell's hypothesis that intake of animal protein may be an important cofactor in liver cancer development. We observed no difference in daily intake of animal protein between liver cancer cases and their matched controls ($P = 0.26$). Campbell also suggested that this "animal protein" effect should be especially pronounced among hepatitis B surface antigen-positive carriers of hepatitis B virus. Again, we did not observe any difference in

Received 6/13/94; accepted 6/15/94.

[1] The abbreviation used is: HBV, hepatitis B virus.